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THE STUDY OF HUMAN MUTATION RATES*

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The focal nature of knowledge concerning the amount and kind of mutation occurring spontaneously in a given species in an understanding of the population genetics of that species is today widely appreciated. Equally appreciated are the difficulties inherent in studying mutation even in material so amenable to genetic manipulation as maize and Drosophila. In the face of these well recognized difficulties, it will seem to some premature, and to others highly speculative, that we should devote a portion of this symposium to the subject of human mutation. To my mind, there are two justifications for so doing. The first is that man is probably not as unfavorable an object for such studies as is commonly believed, and it is high time we appreciated his possibilities in this respect. The second is that when the various scattered studies dealing with human mutation rates are all assembled, there exists a body of data more extensive than is usually appreciated and sufficient to warrant even now our serious appraisal.

For the purposes of this discussion, we shall define mutation as a change in the genetic material resulting in inherited variation. That this definition involves a gross oversimplification of a very involved process is of course obvious. But for our present purposes, we need not enter into the intricate biochemical and chromosomal problems presented by the mutation process, leaving these to such occasions as the most recent Cold Spring Harbor Symposium.

SOURCES OF BIAS IN MUTATION RATE ESTIMATES

It will be well to recognize at the very outset several fundamental biases in the study of human mutation rates. Mutation can be recognized and its frequency measured with reasonable certainty only if it results in a clear-

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cut departure from an antecedent phenotype, a departure whose inheritance can be analyzed in the simple terms of unigenic heredity. But there is ample reason to suspect that the results of mutation at some genetic loci are much more elusive in their phenotypic expression than at others, and that even at a given locus by no means all of the mutations are detected with equal ease. Furthermore, we are not likely to undertake a study of the rate of mutation of a particular gene unless the phenomenon occurs with sufficient frequency to render such a study feasible. In other words, present knowledge of mutation rates is based upon a biased sampling of some of the mutations of some of the many thousands of genes each of the higher plants or animals possesses. We shall return later to the nature of this bias.

The above described source of bias is common to all studies of mutation, no matter what the species involved. The second type of bias which we should recognize at the outset is more specific for studies involving man. Identical, or shall we say clinically indistinguishable, phenotypes may arise as a result of mutation at any one of several loci. In such organisms as Drosophila, it is possible to allocate to each locus its share in the total picture by the appropriate genetic analyses. This is not the case for man. We can never be certain in man that the sudden appearance of a given trait is always due to mutation at the same locus, or, in sporadic cases of certain diseases, that it is due to germinal rather than somatic mutation. However, on the basis of comparative genetics and developmental physiology, we can at least select for study specific phenotypes which with a high degree of probability are due to change involving a particular locus. Thus, most if not all pedigrees of achondroplasia, or hemophilia, or retinoblastoma are probably due to mutation at the same locus, whereas this is not so for less specific entities such as hypertension or diabetes.

It is convenient in mutation rate studies to distinguish between recessive, incompletely recessive, and dominant mutation, each of these types of mutation presenting certain problems of rate estimation not raised by the other two. This distinction as applied to any particular gene is actually often quite artificial, being based on the present state of our knowledge rather than on any sound theoretical reason. There is increasing evidence that completely recessive mutation is as rare as completely dominant mutation. In man more and more genes are being found to have some effect when heterozygous (Neel, 1947, 1949), and it seems probable that in time a majority of human genes will be classified as incompletely recessive. But the present distinction into these three categories is a useful working arrangement as long as we bear in mind that it is often based on incomplete knowledge and is arbitrary in the sense of delimiting intergrading regions in a spectrum of dominance relationships.

Basic to all mutation rate estimates is accurate information concerning the frequency in the population under study of the phenotype associated with the gene (or genes) in question, and the selection pressures which bear upon this phenotype. The time may come when each state will estab-

lish central registries of various congenital and chronic diseases. As a matter of fact, there is already available partial data of this type for many states. Whatever the various pros and cons of such a step may be, there is no gainsaying the fact that incidentally it would be a great aid to geneticists interested in population problems. Only one who has attempted to derive an accurate incidence figure for an uncommon inherited disease can truly appreciate the approximate nature of many of our figures, and the difficulties involved in being reasonably certain that in a given population sample one has located *all* persons with a particular trait. The determination of "selection pressures" is even more uncertain. Where a trait is lethal or near lethal, the problem is not so difficult, and most of the studies to date have dealt with such characteristics. But when we deal with characteristics less distinctly deleterious, we encounter a very involved issue. The gene frequencies of today are by and large determined by the selective pressures of one or two or even more hundreds of years ago, the outstanding exception to this generality involving dominant genes strongly selected against. It is a brave man who will attempt to estimate the coefficient of selection for alcaptonuria or hyperuricemia or essential hypercholesterolemia two hundred years ago.

THE STUDY OF DOMINANT MUTATION

With these necessary preliminary remarks behind us, we may turn now to the actual estimation of mutation rates, considering first a dominant mutation which occurs so rarely, or whose corresponding phenotype is so strongly selected against, that the trait is seldom encountered and the occurrence of homozygous individuals may be disregarded. One has first to select a suitable area for study and then to locate all families in this area in which one or more individuals with the trait determined by the mutation in question have been born during a given period. By a comparison of the total number of affected persons with the total number of births in that area during that same period, an accurate prevalence figure is derived.[1] At this point two alternative procedures are possible. One can determine which of the affected persons are the only such occurrence in their family, and presumably owe their origin to mutation. The actual mutation rate for non-sex-linked genes, since man is diploid, is one-half the prevalence at birth of isolated cases of the trait in question. Or alternatively, one can calculate the effective fertility of affected persons in terms of per cent of normal and then, assuming equilibrium, calculate how frequently new cases must arise by mutation each generation in order to maintain a constant proportion of persons with the trait in the population. The appropriate formula is

$$m = (1 - f)\, q \qquad (1)$$

[1]This procedure must be slightly modified in case the trait in question is not immediately apparent at birth but develops somewhat later.

where

m = the mutation rate per gene per generation
f = the relative fertility of individuals heterozygous for the particular gene under consideration, and
q = the frequency of the gene, in this case, ½ the total prevalence of the trait.

The first procedure has the advantage that it involves no assumptions concerning effective fertility, but the disadvantage that the estimate so derived may be too high because included in the material are some individuals who owe their trait to developmental accidents and poorly understood environmental factors rather than mutation, and other individuals in whom the trait is the result of somatic mutation. The latter possibility is particularly to be considered where the manifestations of the disease may be restricted to a portion of the body, and perhaps only one side at that. (See below.) A recent study on retinoblastoma will serve as an example of the first procedure (Neel and Falls, 1951; Falls and Neel, 1951). In the state of Michigan between 1936 and 1945 there occurred 1,054,985 births, of which 53 are known to have resulted in children who developed the malignant ocular neoplasm known as retinoblastoma. This trait appears to be due to a dominant autosomal gene of high penetrance. Two of these children were identical twins, and biologically speaking, reduce the number of affected persons to 52. Of these, 49 were isolated cases within the family, and presumably the result of mutation. The high proportion of isolated cases is thought to reflect the very considerable death toll associated with the disease and the decreased fertility of the blind. The rate of mutation of this gene may at a first approximation be placed at

$$\frac{1}{2} \times \frac{49}{1{,}054{,}985} = 2.3 \times 10^{-5} \text{ per gene per generation.}$$

There is one major and a number of minor sources of error in this estimate. The minor sources, which have been discussed elsewhere (Falls and Neel, 1951), include incomplete ascertainment of the trait in the population, the early death of some children who might if they had lived have developed the tumor, improper diagnosis, etc. These might be termed technical difficulties. The major source of error is of a different nature. It revolves around the assumption, touched on above, that all cases of the disease are due to a germinal mutation at the same genetic locus. Although it is impossible to determine whether mutation at several different loci may result in a tendency to develop an identical neoplasm, it is possible to explore the extent to which somatic mutation enters into the picture and is responsible for an overestimate of the germinal mutation rate[2]. Retinoblastoma when it occurs may affect only one or both eyes. In families where there occurred multiple cases of the disease, the inci-

[2]Somatic mutation is mutation in a somatic rather than a germ cell. It results in a sector of mutant tissue which may or may not, depending on the stage of development at which mutation occurred, include one or both gonads.

dence of bilateral involvement has been, in our experience, 46 per cent, whereas amongst children who had no affected relatives, the incidence of bilateral retinoblastoma was 36 per cent. In other words, there was 10 per cent more bilateral disease among children who were definitely heterozygous for the gene than among children who were assumed to be heterozygous and some of whom actually might be only sectorial mutants. Accepting for the moment the significance of the difference and the fact that it is due to the inclusion in the sample of some individuals who owe their disease to somatic rather than germinal mutation, we can multiply 49, the number of isolated cases observed in our ten-year period, by an approximate correction factor, which in this case is .78, and obtain 38 as the number of affected persons who owe their disease to germinal mutation. This correction reduces the mutation rate estimate from 2.3×10^{-5} to 1.8×10^{-5}.

This approximate correction factor is obtained from the formula

$$cf = \frac{b}{c} \tag{2}$$

where cf = the correction factor

b = percentage of bilateral disease in sporadic cases

c = percentage of bilateral disease in cases where two or more involved persons have occurred in a single family.

This correction assumes that the proportion of bilateral cases in those instances where the disease is due to heterozygosity for the retinoblastoma gene is the same in isolated instances as it is when there are multiple occurrences within a family. Since unilaterally affected persons are more apt to reproduce than bilaterally, it may be that those individuals with the disease who reproduce tend also to have genetic modifiers partially suppressing the manifestations of the gene, in which case the assumption is incorrect.

In passing, it should be pointed out that the findings with respect to retinoblastoma are scarcely such as to permit generalization concerning the extent to which somatic mutation complicates other human mutation rate estimates, both because of the highly specialized (non-mitotic) nature of many of the tissues of the eye and the nature of the trait (cancer) under study.

As noted earlier, completely dominant genes in man are probably quite uncommon. Individuals homozygous for completely dominant genes will have an appreciable frequency in any given population only if the coefficient of selection against the corresponding phenotype is relatively small or zero. Formula (1) may also be used to calculate the rate of mutation to a completely dominant gene which is sufficiently common that homozygous individuals are no longer a negligible factor, if the symbols are redefined so that f is now the coefficient of selection against either homozygote or heterozygote, and q, the gene frequency, is now

$$1 - \sqrt{\text{the frequency of individuals neither homozygous nor heterozygous for the gene in question.}}$$

THE STUDY OF COMPLETELY RECESSIVE MUTATION

Turning now to estimates of the rate of mutation of recessive genes, we find that the direct approach is no longer feasible, and that we must rely entirely on the indirect method. That is to say, a completely recessive gene arising in consequence of a mutation cannot be detected with certainty at the time of its origin. Instead, a newly arisen recessive is usually transmitted through several or many generations of heterozygotes who, at least in the present state of our knowledge, appear phenotypically normal, until finally, from the marriage of two such heterozygotes, there results a homozygous person who is evidence for the occurrence of mutation at some time in the past. In a population at equilibrium, the rate of mutation of a completely recessive gene is approximated by the formula

$$m = (1 - f)\,[\alpha q + (1 - \alpha)q^2], \tag{3}$$

where

m has the same significance as previously,
f = the relative fertility of individuals homozygous for the gene under consideration,
q = the frequency of the gene, and
α = the mean coefficient of inbreeding.

Since the expression $[\alpha q + (1 - \alpha)q^2]$ is the prevalence in the population of the trait determined by homozygosity for the gene in question, it is not necessary in calculating mutation rates to determine either α or q.

Haldane (1949) has pointed out the two major objections to the uncritical use of this formula. The first is the assumption of complete recessivity. If heterozygosity for a given gene confers upon its possessor even a very slight selective advantage, this will, because of the relative frequency of heterozygotes as compared to homozygotes, offset a considerable disadvantage which the same gene may confer upon the homozygote. Conversely, if a gene which in the homozygous condition exerts a deleterious effect also has a very slightly harmful effect on viability or fertility when heterozygous, the rate of mutation necessary to maintain the gene frequency is greatly increased over that necessary to maintain a similarly defective homozygote whose heterozygote is truly neutral. That a not inconsiderable proportion of all recessives may have appreciable effects from the standpoint of natural selection when heterozygous was suggested by the work of Masing (1939), Berg (1941), and Wright, Dobzhansky, and Hovanitz (1942) and recently demonstrated more conclusively by Stern and Novitski (1948) and Muller (1950). In man, the determination of small selective advantages or disadvantages—say something under 5 per cent—is at the present time only rarely feasible, and yet, positive selective factors of that magnitude due to heterozygosity for a gene which in the homozygous condition entirely or largely prevents reproduction, will, if the gene frequency is less than approximately 0.05, usually outweigh in importance from the standpoint of natural selection the effects of homozygosity for

the same gene. This is because under these conditions the number of heterozygotes is so much greater than the number of homozygotes.

The approximate formula necessary to correct for incomplete recessivity has been supplied by Wright (1929) in the form of

$$q^2(1-2h) - q(2-3h+hm) + 1 - h - \frac{m}{1-f} + hm = 0, \tag{4}$$

where m, f, and q have the same meaning as previously, and h is a factor which multiplied by $(1-f)$ results in the selective disadvantage of the heterozygote. This equation may be restated in terms of m as follows:

$$m = \frac{q^2(1-2h) - q(2-3h) + 1 - h}{qh + \frac{1}{1-f} - h} \tag{5}$$

The second major objection to the calculation of the rate of mutation of recessive genes is the error introduced by the assumption of what the geneticist terms "population equilibrium." With rare possible exceptions, human populations are definitely not at present in genetic equilibrium. With increased population movement and changing social customs, there has almost certainly been in the past several centuries a decrease in the coefficient of inbreeding of most communities. One consequence of this will be an increase in the frequency of deleterious recessive genes, until a new equilibrium point is reached. Some hundreds of years are required to reach this new equilibrium. During this period of increase, the ratio of heterozygous: homozygous individuals which the Hardy-Weinberg law postulates will be disturbed, with a relative excess of heterozygotes. Consequently, a mutation rate estimated on the basis of the frequency of homozygous individuals will tend to be too low. A single illustration will suffice to make the point. In both Japanese and European populations, the recessive genes responsible for albinism and congenital total color blindness appear by a method of estimation to be mentioned below (formula 6) to have frequencies (q) in the neighborhood of 0.005, while those responsible for infantile amaurotic idiocy and ichthyosis congenita appear to have frequencies of approximately 0.0015 (Neel, Kodani, Brewer, and Anderson, 1949). Albinism and congenital total color blindness may somewhat arbitrarily be assigned f values of 0.5, whereas for infantile amaurotic idiocy and ichthyosis congenita the f value is more like 0.0. Referring to formula (3), we see that we may calculate the approximate mutation rate necessary to maintain this situation if we know α, the mean coefficient of inbreeding. Here a complication arises. For European populations, α is in the neighborhood of 0.001 (Haldane and Moshinsky, 1939). But for Japanese populations, α is probably more like 0.006 (Neel, Kodani, Brewer, and Anderson, 1949). If we use the European values of α, the calculated mutation rates for these genes are 1.5×10^{-5} and 4×10^{-6}, whereas with the Japanese values, m becomes 2.8×10^{-5} and 1.1×10^{-5}. In other words, the Japanese mutation rate appears to be twice that of Europeans with respect to these

four genes. While the possibility of such a difference cannot be denied, it seems at least as reasonable to postulate that European populations some two or three-hundred years ago, prior to the Industrial Revolution and the phenomenal growth of cities, had α values as high and perhaps even higher than those of present day Japan. If this is true, then mutation rate estimates based on Japanese material would be the more accurate, although these too may be underestimates because of the probability of a similar but less marked relaxation of inbreeding in Japan in recent times. Unfortunately, there seem to be no published data concerning consanguinity rates 100 years ago in either Europe or Japan, although the possibility that such data could be derived from a study of church records, as Maia (unpublished) has recently done in South America, should not be overlooked.

As noted earlier, because of practical difficulties the determination of the frequency of rare recessive traits, a procedure necessary to gene frequency estimates, is fraught with error. Fortunately, there is an alternate approach to the estimation of q. As numerous authors have pointed out (Lenz, 1919; Weinberg, 1920; Dahlberg, 1938; Hogben, 1946), q may be calculated if the proportion of first-cousin marriages among the parents of persons with the trait in question and among the population as a whole is known, according to the formula

$$q = \frac{c\,(1-k)}{16k - 15c - ck} \tag{6}$$

where k = the proportion of first-cousin marriages among the parents of persons with the trait in question, and

c = the proportion of first-cousin marriages among the population as a whole.

The use of this formula implies that first-cousin marriages are contracted at random in the population, an assumption probably contrary to fact in most communities, but the formula is still useful as yielding a first approximation to the true value.

The two above discussed theoretical objections to calculating the rate of mutation of recessive genes are of such moment that all the calculated mutation rates of recessive or incompletely recessive genes must be regarded as highly tentative. However, so long as the possible sources of error are kept clearly in mind, the calculations are of value in helping fix our sights on the problem.

THE STUDY OF INCOMPLETELY RECESSIVE MUTATION

Finally, as the third class of genes for which mutation rates can be calculated, we may consider those which are incompletely recessive or semidominant, that is, genes which find expression in both the heterozygous and homozygous state, but with milder manifestations in the heterozygote. An approximate formula (5) for calculating the mutation rate of such genes has already been derived. The effects and distribution of two genes which fall in this category have been rather extensively studied. These are the

genes responsible for the sickling phenomenon of red blood cells and for the anemia known as thalassemia (review in Neel, 1951). Both of these genes exhibit restricted racial distributions, the one being confined largely to African Negroes and their descendants, and the other particularly to the inhabitants of regions bordering on the northern and eastern aspects of the Mediterranean Sea and the islands of the Mediterranean, and their descendants. Accurate gene frequency maps are not available for either of these genes, but in Africa the frequency of the gene responsible for sickling is known to vary from near zero in some areas to as high as 0.15 to 0.20 in others, while in the Mediterranean regions the frequency of the thalassemia gene ranges between near zero in some areas and a known maximum, according to a recent report from Cyprus (Banton, 1950), of approximately 0.10. Individuals homozygous for the thalassemia gene reproduce so seldom that for purposes of calculation their relative fertility (f) may be set at 0.0. Individuals homozygous for the sickling gene who have been studied in the United States are somewhat less handicapped, their effective fertility probably being in the neighborhood of 0.2. The occurrence of gene frequencies of this order of magnitude where two such deleterious traits are concerned raises some interesting genetic questions. Are these frequencies the result of mutation rates which would put these genes in the category of the "mutable genes" of Drosophila—and if so, why the restricted racial distribution? Or is the rate of mutation within the normal range, and the high incidence due to some positive selective value of the heterozygote which at least in part offsets the low effective fertility of the homozygote—and if so, again why the restricted racial distribution?

The types of data necessary to differentiate between these two possibilities and the available critical evidence bearing on these questions have been discussed elsewhere (Neel, 1951). Thus, one may, on the one hand, investigate the physiology and reproductive behavior of the heterozygote. Individuals heterozygous for the thalassemia gene average two grams of hemoglobin per 100 cc. of blood less than normal (Valentine and Neel, 1948), and the life span of their erythrocytes is definitely decreased (Zuelzer and Kaplan, 1950; Hamilton, Sheets, and DeGowin, 1950; Astaldi, Tolentino, Sacchetti, and Nonanto, 1951). It is difficult to see how such a hematological disturbance can be of selective value. On the other hand, there are some data to indicate that there is a type of reproductive compensation operative, so that marriages of two heterozygotes yield more children than the marriage of normal by normal or normal by heterozygote (Silvestroni, Bianco, Montalenti, and Siniscalo, 1950). How recent a development this reproductive compensation is, and whether it offsets the increased mortality among the children of two heterozygotes, remain to be demonstrated. There is some indirect evidence that the heterozygote for the sickling gene has a decreased life span. No data are yet available concerning the reproductive performance of individuals heterozygous for the sickling gene as contrasted to normal. For neither of these conditions, then, is it yet possible to make statements about the net reproductive efficiency of the heterozygote.

As a second general approach to differentiating between the two possibilities of high mutation rate vs. selection in favor of the heterozygote, one may seek for actual evidence for mutation. In the absence of mutation, homozygous individuals can only arise from the marriage of two heterozygotes. Conversely, if an individual whose phenotype is definitely that of a homozygote is found to have one normal (that is, non-heterozygous) parent, then, after appropriate steps have been taken to exclude, insofar as possible, non-paternity, failures of penetrance, and certain genetic complications, here is presumptive evidence for mutation. During the course of the studies on the sickling phenomenon which have been carried out in our laboratory during the past three years, a number of families which may be interpreted as supplying evidence for the occurrence of mutation have been observed. However, the accumulation of such data proceeds slowly, and it will require some further years before it will be possible to determine whether these apparent cases of mutation occur with a sufficient frequency to explain the relatively high incidence of the disease among Negroes.

Incidentally, in the course of these studies we have come upon a very interesting illustration of how cautious one must be in documenting cases of the apparent mutation of recessive genes. We have now encountered among the families where a child appeared to have sickle cell disease (that is, to be homozygous for the sickling gene), but where only one parent had the sickle trait (that is, was heterozygous), and where repeated tests failed to elicit sickling in the blood of the apparently normal parent and serological studies gave no evidence that the legal father was not the biological, several instances in which electrophoretic studies of the hemoglobin of the apparently normal parent revealed the presence of an abnormality which was then shown to be inherited as if due to a dominant gene. The interaction of this newly discovered gene with a single sickle cell gene, and not mutation, was responsible for the apparent exception to the general rule in these families (Itano and Neel, 1950; Kaplan, Zuelzer, and Neel, 1951).

I cannot escape the conviction that when we fully understand the explanation of the frequencies of thalassemia and the sickling phenomenon we will have made a very significant step toward understanding the factors operative in the differentiation to be observed within the human species.

SUMMARY OF THE VARIOUS AVAILABLE ESTIMATES OF THE RATE OF MUTATION OF HUMAN GENES

In the foregoing, some of the many difficulties inherent in estimating the rate of mutation of human genes have been discussed. It is apparent from this long parade of technical and theoretical pitfalls that a large margin of error enters into the determination of the rate of mutation of any particular gene. Nevertheless, the available estimates must be regarded as sufficiently accurate to fix an order of magnitude. Table 1 summarizes the available data concerning human mutation rates. The estimates given for

TABLE 1

SUMMARY OF ESTIMATES OF THE RATE OF MUTATION OF HUMAN GENES

Classification of gene	Character produced by gene	Mutation rate/ gene/generation	Author
Autosomal dominant	Epiloia	$0.8\text{–}1.2 \times 10^{-5}$	Penrose, 1936
	Chondrodystrophy	$4.2\text{–}4.8 \times 10^{-5}$	Mørch, 1941
	Pelger's nuclear anomaly	8.0×10^{-5}	Pätau and Nachtsheim, 1946
	Aniridia	$> 1.2 \times 10^{-5}$	Møllenbach, 1947
	Retinoblastoma	1.4×10^{-5}	Philip and Sorsby, unpublished
		2.3×10^{-5}	Neel and Falls, 1951
	The Rh loci	5.1×10^{-4}	Reed, in press
Autosomal recessive	Microphthalmos and anophthalmos with or without oligophrenia	$1.0\text{–}2.0 \times 10^{-5}$	Sjögren and Larsson, 1949
	Albinism	2.8×10^{-5}	Neel, Kodani, Brewer and Anderson, 1949
	Congenital total color blindness	2.8×10^{-5}	Neel, Kodani, Brewer and Anderson, 1949
	Infantile amaurotic idiocy	1.1×10^{-5}	Neel, Kodani, Brewer and Anderson, 1949
	Ichthyosis congenita	1.1×10^{-5}	Neel, Kodani, Brewer and Anderson, 1949
	Cystic fibrosis of pancreas	$0.7\text{–}1.0 \times 10^{-3}$	Reed, in press
Autosomal incomplete recessive	Thalassemia	$?4.0 \times 10^{-4}$	Neel and Valentine, 1947
	Sickle cell disease	$?1.0 \times 10^{-3}$	Neel, 1951
Sex-linked recessive	Hemophilia	3.2×10^{-5}	Haldane, 1947
	Pseudohypertrophic muscular dystrophy	1.0×10^{-4}	Stephens and Tyler, 1951

the recessive and incompletely recessive genes are all based on the explicit assumption of no selection on the heterozygote. It seems quite probable that with the passage of time this assumption will in certain specific instances be shown to be incorrect, a development which of course would result in a revision of some of the figures.

A number of other estimates where the data are quite meager have been omitted from the table. For instance, Mongolism, with an incidence of at least 1.5×10^{-3} (Carter and MacCarthy, 1951), has been attributed to a dominant mutation (Kemp, 1944). Mongols of course only very rarely reproduce, so that critical data concerning the validity of this hypothesis are not available, but it is noteworthy that of the three children reported in the literature as being born to Mongols, one was very probably also a Mongol (Lelong et al., 1949). If this hypothesis of the etiology of Mongolism proves correct, then this is the highest human mutation rate of general distribution yet recognized.

Haldane (1948) has suggested that the available estimates of human mutation rates are higher than the species average, because of the selection that enters into these estimates. As the present author has pointed out elsewhere (Neel and Falls, 1951), this is probably true with respect

to genes with clear-cut and striking effects. However, for every mutation with clear-cut and readily appreciated effects there are many more which are either lethal when homozygous or else affect viability and fertility in obscure and poorly defined ways. X-ray experiments suggest that the ratio of visible mutations to dominant and recessive lethals to mutations with ill defined effects is in the neighborhood of 1 : 10 : 20 (Muller, 1950). There are theoretical reasons for surmising that when mutation at a given locus results in clear-cut and striking effects, the rate of mutation at that locus tends to be less than at loci with less clear-cut effects. A corollary of this is that at any given locus mutations with "small" effects may well be more frequent than those which result in more marked departures from normal. In other words, the available mutation rate estimates in man are probably based upon the more frequently mutating among a group of genes which as a whole have a relatively low mutation rate, at least with reference to the types of changes that can be readily studied.

These and other considerations have led Muller (1951) to suggest that the total mutation rate in man per generation may be as high as 0.1 to 0.5, and the present author (1951) to suggest an even higher figure, of the order of magnitude of 1.0. This is to say, each individual, on the average, is postulated to possess at least one mutant gene not present in either of his parents. I am at present inclined to view this as a rather conservative estimate. In arriving at an estimate of 0.1 to 0.5, Muller was influenced by the fact that the results of mutation, by and large, are unfavorable, any given mutant gene usually resulting after few or many generations, depending on how deleterious its effects are, in the death (or "half-death") of the individual who receives it. It seemed to Muller that the figure of $0.1 - 0.5$ represented the maximum that was compatible with the successful survival of the species. There are, however, many factors that combine to make difficult a priori probabilities concerning human mutation rates. In particular, mention might be made at this point of the significance to genetic thought of the concept of multiple coexistent alternative metabolic pathways for a given metabolite within a given cell (cf. particularly Potter and Heidelberger, 1950). That genes frequently act through controlling the presence and amount of particular intracellular enzymes is of course a well established tenet of genetics. Were each metabolite free to follow but a single anabolic or catabolic sequence of enzymatically determined reactions, then a genetically controlled interference with any step in this sequence might, if the metabolite in question occupied a significant role in cell economy, assume the proportions of a limiting factor. With, however, alternative sequences available, the possibilities of intracellular adjustments to metabolic abnormalities are broadened, and by inference the number of mutations which may occur without any one becoming critical is greater. In other words, even at the cellular level, selection may act upon combinations of genes having to do with a particular reaction rather than single genes.

As must be apparent by now, questions of mutation go hand in hand with questions of selection. Our knowledge concerning the operation of selection in various human groups is almost negligible. Here is a fertile field for anthropological investigation. What are the factors that determine survival in primitive societies? If, as Dobzhansky and Montague (1947) maintain, there came a point in man's evolution at which mental ability became the primary asset, with selection in all types of communities toward this end, perhaps the forces which usually operate to hold the rate of mutation at a more or less optimum level for the species have in the case of man been seriously disturbed by the great development of the central nervous system.

Much of our thinking regarding human mutation rates is strongly influenced by what we know about mutation in Drosophila, where the rate of mutation per gene per generation is usually thought to be of the order of 1×10^{-5}, which, with a total gene number of 5,000–10,000, implies a total mutation rate of 0.05 to 0.10. From the standpoint of the "mechanics" of evolution, however, at least two important differences must be recognized between Drosophila and man. The first of these involves number of offspring, the second, the life span. At maximum reproductive rates, a pair of humans might in 25 years possess 6–8 offspring, whereas a pair of flies, averaging ten generations to the year and 50 pairs of offspring each generation, have the potential of 50^{250} pairs, a truly staggering figure and of course far greater than the total number of representatives of the human species that have ever lived. One certainly wonders to what extent, in the face of this enormous difference in reproductive potential, there are grounds for postulating a similarity in mutation rates in terms of gene-generations. Assuming the findings in Drosophila to be more or less typical of shorter-lived forms, must man, in the face of the potentially rapid change in the genetic attributes of his competition, maintain, through a "high" mutation rate, a state of evolutionary plasticity, or, in view of his low reproductive performance, is the premium on the maintenance of a type which in the past has in competition proved successful, with the inference of a "low" mutation rate? I suspect that in the present imperfect state of our knowledge you would find as many to take one side of that question as the other.

At least one anthropologist has had the temerity to face the question of whether selection, working with the building blocks furnished by mutation, can account for the evolution of *Homo sapiens* from Neanderthal man of the Third Interglacial Period (McCown, 1951). As a geneticist I can but admire Dr. McCown's courage and wish that we could supply him with better data concerning mutation.

CONCLUDING REMARKS

In the introduction to this paper it was stated that man is probably not as unfavorable an object for spontaneous mutation rate studies as is genally believed. The preceding consideration of the various difficulties which

arise in the collection and interpretation of the appropriate data will seem to some to render that opening statement unduly optimistic. Let us in closing restore our equilibrium by considering the advantages of human material. Human populations can be enumerated and the characteristics of their members evaluated in a manner seldom possible for animal communities. Family histories, including data pointing to the probable time of origin and mean number of generations of survival of dominant mutations, can be obtained somewhat more readily from man than from Drosophila. The selective value of various traits can be evaluated in man under somewhat more natural conditions than the various rather artificial environments which so often form the background for Drosophila experimentation. There is at the disposal of the investigator seeking to evaluate the significance to the human organism of a given trait a wealth of background biochemical and physiological data such as exists for no other organism.

In closing, I would like, as a geneticist, to indicate where in the study of mutation I see the greatest opportunities for hybridization with the disciplines represented at this meeting. As regards statistics, it has often in the past been uncertain where geneticist ended and statistician began. However, few geneticists are sufficiently familiar with recent developments in the field of population sampling, developments which undoubtedly are pertinent to the estimation of gene frequencies. As regards anthropology, it should be recognized that the anthropologist has a start of many years on the geneticist in the study of populations. It will be interesting to see how he utilizes that experience as he becomes increasingly aware of the significance of the concepts of population genetics to anthropological research.

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GENETIC DRIFT IN A RELIGIOUS ISOLATE: AN ANALYSIS OF THE CAUSES OF VARIATION IN BLOOD GROUP AND OTHER GENE FREQUENCIES IN A SMALL POPULATION

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By general recognition race is now increasingly defined in terms of differing gene frequencies, and the origin of such differences has become of very great interest. Sewall Wright, in a notable series of papers (1931, 1932, 1940, 1943, 1948) has developed the theoretical aspects of the interaction of the four factors which can upset existing gene frequency equilibria and establish new gene frequencies, namely, mutation, selection, migration, and genetic drift. In particular, he has pointed out the limitation upon the effectiveness of mutation, migration, and selection pressure in those populations wherein 4Nv, 4Nm, or 4Ns, respectively, are less than 1 (N = effective size of the population; v = mutation rate; m = net migration rate; s = coefficient of selection pressure). Whenever v, m, and $s < \frac{1}{2N}$, genetic drift sets in. That is to say, gene frequencies oscillate about their equilibria, the oscillations become larger in magnitude as the population size becomes smaller, and the cumulative effect over a number of generations may lead to the elimination of some alleles and the fixation of others, as well as to any intermediate frequencies. Wright (1948) has calculated that this can happen even in populations of an effective size up to 250,000 when the mutation rate is of the order 10^{-6} and when selection and migration are also extremely low. By observation, we know that mutation and migration may quite often be so low as that. It is far more difficult to show that competing alleles are sufficiently neutral to satisfy this condition.

Fisher and Ford (1947, 1950) have studied the fluctuations in frequency of a gene in an isolated population of the moth *Panaxia dominula* L. over a period of eight years, and this study has been extended by Sheppard (1951) to cover four additional years, through 1950. Considerable fluctuations in the gene frequency of the medionigra gene were observed in a population calculated to be too large for such shifts to be attributable to genetic drift. This may be so. Still, to prove that the fluctuations in the frequencies of the alleles at one locus are due to selection rather than genetic drift has no direct bearing on the question whether the fluctuations at some other locus are attributable to the one or the other. If the critical size of

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the population is $\frac{1}{2s}$, then a population (say of 10^4 individuals) which is "enormous" with respect to a gene having a selection value of 0.2 will be "small" with respect to a gene at some other locus for which $s = 0.0001$. The real difficulty, as Sheppard has pointed out, is to be sure that characters of neutral survival value actually are such, especially since survival value undoubtedly, in many cases such as that of the medionigra gene, itself varies from year to year and place to place.

In the application of these concepts to human evolution and the origin of human racial differences, it must be kept in mind that "throughout the greater part of his history the... breeding populations of man appear to have been very small. Peoples at the lower hunter stage of cultural development at the present day rarely if ever attain a breeding population size of 1,000" (Montagu, 1950, p. 329). It is also important to remember that the genetically effective size of a population is characteristically smaller, often very much smaller, than the number of individuals it contains. In a fluctuating population which undergoes periodic reductions in size, N is the harmonic mean of the maximum and minimum sizes of N and is therefore very much closer to the minimum. According to Wright, if N_o and $10^6 N_o$ are the respective minima and maxima in a 7-generation cycle, then $N = 6.3N_o$. Probably many existing human populations are the derivates of once very small genetic isolates which have undergone an explosive increase in size, either at the dawn of agriculture or later. In that case gene frequencies in different races might well reflect the fortuitous results of genetic drift in the original isolates, since in a population with an effective size of 200 an allele would require a selective advantage of 0.01 to avoid the action of drift, and consequently many genes might be effectively neutral.

Numerous examples of divergent gene frequencies in small and relatively isolated human populations are to be found. One of the most extensive studies of this sort of situation is the contribution by Birdsell to the 1950 Cold Spring Harbor Symposium. Birdsell found "inter-isolate fluctuations suggestive of genetic drift" in the native tribes of Australia, which average about 500 persons in size (N, ca. 200) and correspond approximately to genetic isolates. The very plausible attribution of such differences by Birdsell and others (Birdsell and Boyd, 1940; Boyd, 1950; Laughlin, 1950; Montagu, 1950; Dobzhansky, 1950) to genetic drift has, however, been challenged by those who hold that all such differences are more probably attributable to differential selection. The interesting discussion on this point between Cain, Birdsell, and Dobzhansky (Birdsell, 1950) has served to emphasize that genetic drift and selection are not alternative explanations but are factors which may often act in conjunction, as Sewall Wright has constantly maintained. Nevertheless, the conclusion that genetic drift is at all responsible for the observed differences in gene frequencies in the Australian tribes is rendered uncertain by the possibility that selection might operate in these circumstances and be the chief or sole cause of the genetic differences.

What appears to be needed for a clearer solution of this problem is the analysis of a genetic isolate of known size, age, and origin and which in particular shares an environment indistinguishable from that of the major population with which it is to be compared. The situation ideal for study would be that of a genetic isolate interspersed within a larger population, so intermingled that the individuals of the isolate do not differ from those of the general population in any aspect of life except their assortative breeding restrictions. The requirement is to find a type of isolate neither geographic nor economic, since such isolation factors might indeed be correlated with selective factors. Nor is an ethnic isolate very suitable, since in such a case one would presumably have to deal with initial differences in gene frequency as well as with possible differences in environment that might have a selective effect. The very type of isolate desired exists, however, in the communities of certain endogamous religious sects.

RELIGIOUS ISOLATES

A significant study of this type has been made by Sanghvi and Khanolkar (1949), who have reported that in six endogamous groups (castes or subdivisions of castes) in India the frequencies of blood group genes (ABO, A_1A_2, MN, P, and Rh) and of the genes for P.T.C. taste capacity and color vision differ significantly in many intergroup comparisons. Two closely related groups were identical. Of the remainder, "the endogamous groups V.N.B. and C.K.P. differ from all the three other groups and from each other in more than two characters, with a high degree of significance. The group K.B. differs significantly from the groups D.B. and M.K. in only one character, viz. OAB blood groups. Groups D.B. and M.K. do not show significant differences between them for any character." The gene frequency analysis of the Indian population is clearly not properly carried out unless due attention is given to the existence of these endogamous groups. As to the causes of the differences, however, nothing certain can be said. Although the groups studied were all Hindu, the size of only one of them is given, and it is large (3,650,504 persons in the Bombay Presidency). Others are presumably much smaller, but may still number 10,000 persons or more. No historical record is available regarding the origin of these endogamous groups. As to their similarity of environment (nutrition, medical care, etc.), it is by no means clear that extensive differences do not exist. Consequently it seems impossible to conclude that the differences are entirely due to a single variable, and rather likely that both selection and "migration" have played their part. Only genetic drift would appear to be ruled out, except in so far as the isolates were once of small effective size or the genes concerned essentially neutral as to selection.

In the United States similar, but smaller endogamous religious groups exist. In some of these, such as the Amish, the religious community is in fact a microgeographic isolate. In others, the individuals are dispersed in the general community. Their children go to the public schools, and their homes, clothing, food, medical care, and hygiene exhibit no distinctions of

magnitude in contrast to the generality of the population. A comparison of the gene frequencies in such an isolate with those of the surrounding population should indicate more decisively than previous studies the extent to which genetic drift can cause gene frequencies to vary.

This paper is a preliminary report of the study of one such religious isolate, a study conducted by Mr. Charles Hess of the religious community, and by Dr. Milton Sacks, Miss Elsa Jahn, and myself, of the Baltimore Rh Typing Laboratory. Much of the detailed analysis remains to be completed, but enough is apparent at this point to throw some light, I believe, on the effectiveness of genetic drift in altering gene frequencies in human populations.

THE DUNKER ISOLATE

The religious isolate studied is an Old Order "Dunker" (Old German Baptist Brethren) community in Franklin County, Pa. It includes 298 adults and minor children (over 3 years of age and under 21), and a total of about 350 persons when adults are included who, although not members of the religious group themselves, are the children of members and still live in the locality. The effective size of the population may be estimated directly. Among white Americans at the present time, according to Dublin (1951), the average age of mothers at the birth of their children is 26 years. The average age of all fathers is 30 years. Thus 28 years may be taken as the current length of generation. In the Dunker isolate the age distribution of individuals is in good agreement with that of the general population, so that 28 years may be taken as the length of generation in the isolate too. By actual enumeration, persons within the isolate aged 1 to 28 years had 90 parents also belonging to the isolate. This seems to be the best estimate of N. Among living members of the community, both for the generation 1895–1923 and for 1904–1931, there were 84 persons who were parents. Thus a confirmatory value for N of slightly under 100 is obtained. In a population of such size and in the absence of mutation, selection, or immigration, according to Wright, $\frac{1}{2N}$, i.e., in the present case 1/180 or 0.555 per cent of the loci will become fixed or eliminated in each generation. If the number of gene loci in the human species is roughly 20,000, and if no more than 10 per cent of these are variable in the population, then 11 loci would become fixed per generation under the stipulated conditions. The number of loci that would show detectable drift would of course be far greater. Actually, since at least immigration is considerable in the present case, the tendency toward equilibrium will offset the effect of genetic drift to a considerable extent. Instead of fixation one would expect only wide fluctuations around the expected gene frequencies.

It was hoped that we could study 100 per cent of the individuals making up the group, but the time and effort required to get in touch with the last few persons has proved prohibitive. At the present time genetic information has been obtained on 265 persons, of whom 231 are among the 298 persons

coming within the more strict definition of the isolate. The general sampling of the isolate is therefore 77.5 per cent complete, although for individual characteristics the sampling is somewhat less adequate because of the omission or failure of certain tests or items of information. It seems very unlikely, even so, that complete sampling would change the results to any significant degree.

The history of the isolate is briefly as follows. The sect of the German Baptist Brethren was established in 1708 in the Rhineland, Krefeld being a chief center. In 1719 there was a migration of 28 persons from Schwartzenau and the vicinity to Germantown, Pennsylvania. These German Baptists were later joined by others, the number being uncertain but probably several hundred, the majority coming mainly from the same German region, although at least one man was a scion of the nobility of Saxony. In 1881 the sect split into three groups, the largest being the Church of the Brethren, and the next largest the Progressive Church, while a small minority, the most orthodox in retaining the original practices of the German Baptist Brethren, formed the third group. It is this sect, the Old German Baptist Brethren, which provided the community studied as an isolate in the present project. There are at the present time about 3500 members in approximately 55 communities belonging to the sect, located as follows:

Pennsylvania	3	Illinois	2
Maryland	1	Missouri	2
Ohio	16	Michigan	3
West Virginia	2	Wisconsin	1
Virginia	4	North Carolina	1
Indiana	10	Florida	1
Kansas	5	California	3
		Canada	1

Individuals of the sect dress distinctively, but not to the degree practiced by the Amish. The sect does not maintain separate parochial schools, nor does the general way of life of its members differ perceptibly from that of other Americans of the same localities.

It follows that the isolate may be regarded as having been in existence as a religious community in America for over two centuries, with a drastic restriction in the size of the mating group having occurred 70 years ago. The religious community in Franklin County, Pennsylvania, now comprises some 20 extended families, each composed of one to more than a dozen families. A tabulation indicates that over a period of 3 generations approximately one-fourth of the persons reaching adulthood have left the religious group in each generation. These losses are compensated in the most part by the large sizes of the families, so that the size of the group has remained quite stable. There are relatively few childless marriages. In the generation 1895–1923, 85 per cent of persons now living are parents, and only 2 per cent were married but childless. In the generation 1904–1931, only 73.6 per cent of those now living are parents, but of course this lower proportion is to be expected because more persons in the group are young and either unmarried or very recently married. The sizes of the families in one extended family

were 5.4, 5.25, 3.78, and 3.2 children per family in succeeding generations. In spite of the decline, this is even now well above the replacement level of 2.83 (Dublin, 1951).

A tally of all known marriages of members born since 1850 yields the following information about gene flow into the isolate. About 64 per cent of the members of the isolate marry other members of the group (48% of all marriages, A). The frequency of endogamous marriage has remained surprisingly constant over the past three generations. About 9 per cent (13% of all marriages, B) marry individuals of other communities of the sect, mainly from the adjacent community in Frederick County, Maryland. Nearly 27 per cent of the members have married non-members, 10 per cent with those who remained non-members (15% of all marriages, C) and 16.5 per cent with persons who entered the community as converts (24% of all marriages, D). With very few exceptions, the marriages with non-members who remain such represent losses to the isolate rather than gains. Hence the maximum gene flow into the isolate is $B + D/2(A + B + D)$, or 22.0 per cent. In actuality this is diminished by the degree to which the contributing communities of the sect or locality resemble the isolate in composition by corresponding deviations from the general average of the population of the United States. This reduction might well be as much as 8 per cent. Dr. Sewall Wright (by letter) has kindly provided a rough estimate of m, the gene flow into the isolate, by another method. This is based on the calculation of the inbreeding coefficient $\left(F = \frac{\sigma_q^2}{q_\tau(1 - q_\tau)} = \frac{1}{4Nm + 1}\right)$ from the gene frequencies in the Dunker isolate (q) and in the U. S. population (q_τ) for the six genes I^A, I^B, I^O, *M*, *r*, and *dbt*. The estimate of F is .0254, from which it follows that $Nm = 9.3$ and m is about .10. This appears to be of about the order of magnitude expected from the previous considerations. The gene flow into the isolate will therefore be regarded as amounting to 10 or 15 per cent per generation.

The genes lost to the isolate by emigration might be considered to represent a random sample were it not for the effect of the emigration of family units in addition to single individuals. About 8 per cent of the married couples moved away from the geographic area covered by the isolate. Most of these emigrants were young, their families still in the future at the time of leaving. Thus for these cases too the loss to the isolate was random. The non-random losses must be few. To illustrate: within the isolate at the present time there are 25 persons with distal hyperextensibility of the thumb. Of these, no less than 10 belong to one extended family, comprising one couple of grandparents and the families of their five children. Had this entire family moved out of the isolate, the frequency of the doublejointed trait mentioned would be shifted from 16.8 per cent to 12.6 per cent, and the difference from the general population in this respect would be even more striking than it is (see table 5).

Inasmuch as the isolate has remained small and rather stable in size since 1881 in spite of high fecundity, it is obvious that the loss of indi-

viduals through their removal to other regions or through their failure to remain in the sect has been considerable. The maintenance of the group as a genetic isolate has thus depended not so much on the failure of marriages with outsiders to occur, as rather upon the characteristic exclusion from the group of those who do marry outside. The equilibrium is maintained by high fecundity. Thus in each generation the influx of approximately 10 or 15 per cent of genes from the general population, an influx which tends to make gene frequencies in the isolate more like those of the general population, is opposed in part by the effect of genetic drift resulting from the chance exclusion of approximately 10 per cent of the genes within the isolate. The genetic drift resulting from accidents of sampling because of the small effective size of the population (N) appears therefore in this community to be compounded of three elements: (a) the accidental composition of the original community in Franklin County; (b) the effects in each generation of the random sampling of gametes in a very small population; and (c) the chance exclusion of genes borne by those who leave the community.

THE GENETIC CHARACTERS ANALYZED

The primary criteria for characters suitable for the analysis of gene frequencies in a human population may be stated, to paraphrase Boyd (1950), as follows:

> A useful anthropological character must be inherited according to a known mechanism, preferably simple. It must be accurately, and preferably easily, classifiable. It should be relatively stable and non-adaptive. It must be relatively common, not rare. It must vary in frequency in different populations. If it is morphological rather than physiological, and if it is a feature of parts that endure after the death of the individual, i.e., if it is skeletal, so much the better.

The number of such traits known at the present time is decidedly limited, and when one is further limited by lack of reliable data as to frequency in one or both of the general populations (West German and United States) to be compared with the isolate in question, the restriction is indeed severe. In the present study the traits used were the following: ABO blood groups; MN blood groups; Rh blood groups; Middigital hair; Distal hyperextensibility of the thumb; Ear lobes; Handedness. It was intended also to include taste capacity for phenylthiocarbamide (PTC), but owing to an unfortunate technical error a test solution was used that is below the sensory threshold for even the majority of genetic tasters, and for the present this very useful trait must be omitted. The others will be discussed in order.

The ABO Blood Groups: Samples of blood cells were collected from the individuals, diluted in physiological saline solution, and transported to the Baltimore Rh Testing Laboratory for ABO, MN, and Rh typing. Because of unavoidable delays, some samples became hemolyzed en route, and since it was not always possible to replace these samples, the total number of tested individuals is below the total number of individuals from the isolate who supplied genetic information for the survey. Table 1 presents the fre-

TABLE 1

FREQUENCIES OF ABO BLOOD GROUPS

	No.	A	B	AB	O	
(1) W. Germany* (Duisberg)	5,036	2,245	504	237	2,050	
		44.6%	10.0%	4.7%	40.7%	$\chi^2 = 25.47$
(2) Dunker Isolate	228	135	7	5	81	
		59.3%	3.1%	2.2%	35.5%	$\chi^2 = 42.14$
(3) U. S. A.† (New York City and N. Car.)	30,000	11,840	3,350	1,250	13,560	
		39.5%	11.2%	4.2%	45.2%	D.F. = 3
	(1) and (2)	P = nearly .001	P = nearly .001	P = .10–.05	P = .30–.20	$P \ll .001$
	(2) and (3)	$P \ll .001$	P < .001	P = .20–.10	P = .05–.02	

GENE FREQUENCIES

	W. Germany	Dunker Isolate	U. S. A.
I^A	.2862	.3778	.2583
I^B	.0743	.0253	.0409
I^O	.6395	.5969	.7008

*Data of Rinkel (from Boyd, 1939).
†Combined data of Tiber and Snyder (from Boyd, 1939).

quencies of the four major ABO blood groups in the isolate in comparison with the frequencies in West Germany and in the United States, taken from Boyd (1939). It is at once apparent that whereas the frequencies in West Germany and in the United States are very similar, those found in the isolate are very different from either. As the χ^2 values show, the deviation is highly significant, and the partition of χ^2 shows that this is due to a marked increase in the frequency of type A and a decrease in that of type B (and AB). The frequency of type O is probably also significantly lower in the isolate than in the U. S. population. The lower portion of the table gives the calculation of the gene frequencies for the three alleles I^A, I^B, and I^O, according to Fisher's new formulae (see Race and Sanger, 1950, p. 21). No combination of values of the frequency of I^A as high as that in the Dunker isolate, nor of I^B as low, have ever previously been reported for a group of Western European origin. One must search among American Indians, Polynesians, or Eskimos to find the like.

The individual origin of the 7 individuals of type B and the 5 of type AB throws added light on the low frequency of the I^B allele in the isolate. Two of type B and one of type AB are children of others in the group of 12. Three of type B and one of type AB are members from another community who entered the isolate by marriage. Two of type B and two of type AB entered the group from the local community by marriage. Of them all, therefore, only one person, of type AB, is of parents both of whom were born in the isolate. The father of this person, presumably of type B or AB since his wife was found to belong to group A, was among those from whom samples of blood were not obtained. It thus appears that the gene I^B had virtually disappeared from the isolate prior to the recent reintroductions from outside.

Subtyping for A_1 and A_2 was also carried out, although not shown in the table. Of 140 type A or AB individuals, 128 were found to be A_1 or A_1B; 12 were A_2 or A_2B. The ratio of A_2/A_1 is given by Boyd (1939) as 0.20 to 0.23 in W. Germany. Values for the United States are quite variant, Wiener and Rothberg (Wiener, 1935) finding 0.48 in 440 individuals typed in New York City, whereas Landsteiner and Levine (1930) obtained a ratio of 0.136 in 194 individuals in the same city. At any rate, the ratio in the United States is probably not very different from that in Western Europe, which is in general above 0.20. The ratio for the Dunker isolate of A_2/A_1 is 0.09. This is significantly different from the combined values for W. Germany obtained in Heidelberg and Kōln ($\chi^2 = 7.43$; D.F. = 1; $P < .01$).

The data presented for the ABO blood types show that whereas in the general population of the United States, in comparison with W. Germany, type O has increased somewhat, no such increase has occurred in the isolate. Instead, type A shows a significant increase, particularly in subtype A_1; and type B has reached the vanishing point. It is worth noting in particular that in the isolate the most extreme departure from the expected gene frequencies (a reduction by two-thirds) applies to the allele which has the lowest frequency in Western Europe and America, namely, I^B, and the least departure from expectancy applies to the allele which is most common,

namely, I^O. This would seem to be expected from the combination of genetic drift and the lower reliability of estimates of small frequencies. On the other hand, there is no reasonable ground for supposing that within the isolate selection can be acting strongly in favor of I^A while in the general United States population the frequency of I^A has been reduced below that which prevails in West Germany.

The MN Blood Groups: In table 2 are given the frequencies of the M, MN, and N groups and the calculated gene frequencies of *M* and *N*. Again it is apparent that the frequencies are significantly different, *M* being in-

TABLE 2

FREQUENCIES OF MN BLOOD GROUPS

	No.	M	MN	N	
(1) W. Germany* (Bonn, Frankfurt am Main, and Köln)	6,800	2,028	3,396	1,376	
		29.85%	49.9%	20.2%	$\chi^2 = 23.68$
(2) Dunker Isolate	229	102	96	31	
		44.5%	41.9%	13.5%	
(3) U. S. A.† (Brooklyn, New York City, and Columbus, Ohio)	6,129	1,787	3,039	1,303	
		29.16%	49.58%	21.26%	
					D.F. = 2
		$P \ll .001$	$P = .10\text{–}.05$	$P = .05\text{–}.02$	$P \ll .001$

GENE FREQUENCIES

	W. Germany	Dunker Isolate	U. S. A.
M	.548	.655	.540
N	.452	.345	.460

*Combined data of Crome, Laubenheimer, and Laves (from Boyd, 1939).
†Combined data of Landsteiner and Levine, Wiener, Herman and Derby, and Hyman (from Wiener, 1935).

creased and *N* decreased by approximately 10 per cent. One must look among the Finns, Russians, and Caucasians, or in the Near East for frequencies comparable to these in the isolate. These *M* and *N* alleles seem particularly suitable for the purpose of the present study, for their almost equal commonness in nearly all human populations implies either that they are selectively neutral, or that the heterozygote is superior to either homozygote, or that a condition of balanced polymorphism prevails, with now *M* and now *N* superior to the other. The considerable decline in the frequency of heterozygotes in the isolate, compared with the frequencies in either of the base populations, rules out the possibility of the superiority of the heterozygote; and the identity of general environment of the isolate and the U. S. general population at least greatly weakens the likelihood of balanced polymorphism. Genetic drift is the remaining explanation.

TABLE 3

FREQUENCIES OF RH BLOOD TYPES

	No.	rh	rh′	rh″	rh′″	Rh_0	Rh_1	Rh_2	$Rh_{1,2}$	
(1) England*	2,000	307	16	19	....	42	1,062	279	275	Actual
		15.10%	0.77%	0.94%	0.02%	2.06%	53.40%	14.08%	13.63%	Theor.
(2) Dunker Isolate	206	23	....	....	....	2	119	32	30	
		11.15%				0.97%	57.9%	15.5%	14.5%	$\chi^2 = 7.01$
(3) U. S. A.† (N. Y. C.)	8,317	1,204	89	47	1	187	4,456	1,225	1,108	
		14.48%	1.07%	0.56%	0.01%	2.25%	53.58%	14.73%	13.32%	D.F. = 4
		$\chi^2 = 2.0(1,2)$ P = .20–.10		$\chi^2 = 4.32$–4.51 P = .05–.02						P = .20–.10

*Combined data of (a) Fisher and Race, and (b) Race, Mourant, Lawler, and Sanger (from Race, Mourant, Lawler, and Sanger, 1948).
†Combined data of (a) Unger, Weinberg, and Lefkon, and (b) Wiener (from Race and Sanger, 1950).

The Rh Blood Groups: The frequencies of the Rh types are given in table 3. No testing for the alleles C^w, C^u, c^v, or D^u was carried out. Not only were testing sera unavailable, but in any case no data for the frequencies of these alleles in the base populations are available.

In contrast to the ABO and MN groups, the Rh frequencies in the isolate present a pattern that conforms closely to the standard for England and the United States. (No frequencies for the subtypes in any German population could be located.) The only deviation of possible significance is found by grouping the rarer types rh′, rh″, and Rh_o. There seems to be a reduction in these rare types within the isolate. There is a small decrease of type rh, but the data are not extensive enough to indicate whether this has significance or not. The commoner types may have increased slightly within the isolate.

The most significant aspect of these data is perhaps the indication that even in an isolate of size sufficiently small to allow genetic drift to operate, still only some of the genes drift in frequency. This is again in accord with theory.

TABLE 4

FREQUENCIES OF MID-DIGITAL HAIR TYPES

	No.	Md_o	Md_1	Md_2	Md_3	Md_4	Others	
(1) Dunker Isolate	234	123	44	23	28	5	11	
		52.5%	19.7%	9.8%	11.9%	2.1%	4.9%	$\chi^2 = 72.8$
(2) Baltimore White	541	146	65	91	146	55	38	
	(56% ♂♂)	26.99%	12.01%	16.82%	26.99%	10.17%	7.02%	
								D.F. = 5
	χ^2	30.8	5.3	5.4	16.4	13.5	1.4	$P \ll .001$
	P	$\ll .001$	.05–.02	.05–.02	$< .001$	$< .001$	.30–.20	

Middigital Hair Types: In table 4 are presented the frequencies for the five main types of middigital hair pattern, supposed to be inherited through a series of multiple alleles, with dominance in ascending order from Md_o to Md_4. Regrettably, no data on middigital hair patterns from any German population seem to have been compiled. Our own control series represents frequencies from Whites of Baltimore, Maryland, from a study by Mr. Henry Plaine, to whom thanks are due for making these data available. The frequency of expression of middigital hair is higher in males than in females. In the Baltimore series, data were taken mainly from high school students, of whom a majority (56 per cent) were males. In the Dunker isolate, because of the greater difficulty of getting in touch with adult males than with adult females or children, there was a preponderance of females (55.4 per cent). Even with correction for the sex difference, the two distributions yield a χ^2 of 65, which for D.F. = 5 gives a P value $\ll .001$. The partition of χ^2 indicates a highly significant increase in the frequency of fingers without hair on the middigital segment, counterbalanced by a

TABLE 5

FREQUENCY OF DISTAL HYPEREXTENSIBILITY OF THE THUMB

	No.	Dht	dht/dht	
(1) Dunker Isolate	149	124	25	
		83.2%	16.8%	$\chi^2 = 4.36$
(2) Baltimore Whites	895	674	221	
		75.3%	24.7%	D.F. = 1

P = .05–.02

significant decrease in frequency of individuals with hair in this segment on three or four fingers.

Distal Hyperextensibility of the Thumb: This trait has recently been studied by Glass and Kistler (unpub.) and has been demonstrated to be due to a Mendelian recessive with a gene frequency of .496 and a penetrance of 96.5 per cent. Inclusion of examination for this trait was begun in the study of the Dunker isolate only well along in the work, so that the number of individuals scored is much below the total examined for other traits. In table 5 the results are given. The frequency of this type of double-jointedness is significantly lower in the isolate than in the Baltimore white population studied (P = .05–.02). The most striking finding is not indicated in the table, namely, that of the 25 individuals with the recessive trait, no less than ten belong to a single family (three generations) in which both parents, and consequently all children, were affected. This family is shown in the pedigree chart, figure 1. It follows that the frequency of the gene *dht* in the remainder of the families making up the isolate must be even lower than the frequency .41 which is the square root of the frequency of the trait.

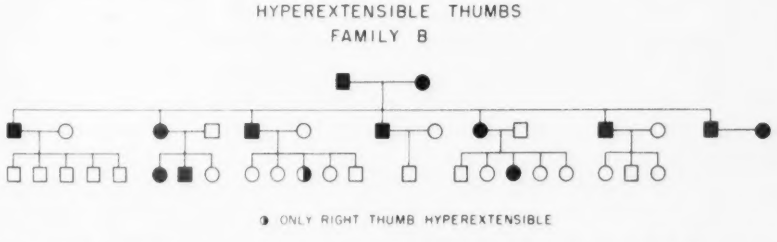

FIGURE 1. Pedigree of a large family showing typical recessive inheritance of distal hyperextensibility of the thumb. Ten of these individuals belong within the isolate studied, and comprise 40 per cent of all the individuals within the isolate who manifest the trait.

Ear Lobes: The control series from Baltimore is not as representative of the surrounding U. S. population in Southern Pennsylvania as perhaps might be wished, but it is all that is available at present. The data indicate a

TABLE 6

EAR LOBES

	Attached	Unattached	Total
Dunker Isolate	61 25.3%	180 74.7%	241
Baltimore Whites	154 40.5%	226 59.5%	380
$\chi^2 = 15.2$	D.F. = 1	$P < .001$	

considerable and significant difference between the two populations in this minor anatomical trait, which is regarded as a single gene difference with free lobes dominant over attached lobes.

Handedness: The frequency of lefthanded plus ambidextrous individuals in the Dunker isolate is 17/243, or 7.8 per cent. This does not appear to be significantly different from the frequency of lefthandedness in the general population. Rife (1945) gives a summary of pooled data for the U. S. population: 93 per cent righthanded; 3 per cent ambidextrous; 4 per cent completely lefthanded. The total of 7 per cent agrees very closely with the frequency in the Dunker isolate, so that in this characteristic, which may well be subject to selection, no deviation is apparent.

DISCUSSION

It is sufficiently apparent from this study that in isolates of about 250 to 350 individuals, and in which N is under 100, considerable deviations in gene frequency occur, both in comparison with the frequencies in the population of origin of the isolate and also with those in the surrounding population. These genetic differences can develop and be maintained, in spite of a gene inflow of 10 or 15 per cent per generation, when promoted by chance sampling of the population in the accidental composition of the original individuals, by the random sampling of gametes in a small population, and by the chance exclusion of genes borne by those who elect to leave the isolate. The study has included analyses of 7 loci, at 3 of which there occur series of multiple alleles that to some extent can vary independently. At 5 of these loci variations in gene frequencies have occurred that are most reasonably attributable to genetic drift. Considering the degrees of freedom among the multiple allelic series, there are in all 15 degrees of genetic freedom between alleles at the 7 loci in the analysis. Eleven of these manifest deviations from the base populations significant at the 5 per cent level. Considering the fact that the traits studied are in all cases common and considering also the homogeneity of environment of the individuals comprising the isolate and those in the surrounding American population, it seems unnecessary to attribute these divergences to some hypothetical influence of selection. In fact, the very loci where selection most demonstrably operates, namely, the Rh and handedness loci, are those where no appreciable drift appears to have occurred.

The conclusion to be derived from the study, that genetic drift can in fact determine gene frequencies to a considerable extent in small human isolates, is still a tentative one. The present analysis should serve chiefly to call attention to a method of attacking the problem and to the existence near at hand of genetic isolates particularly suitable for its investigation.

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EVIDENCES OF AMBIDEXTERITY AND LATERALITY IN THE SEXUAL BEHAVIOR OF CERTAIN POECILIID FISHES

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During courtship behavior in several species of poeciliid fishes, the gonopodium of the male swings to both the right and left sides. First observed by FitzGerald (1872) in Poecilia, this interesting aspect of courtship has been similarly observed in Gambusia, Heterandria, Glaridichthys (now = Phalloptychus and Cnesterodon), Giradinus, Lebistes, Xiphophorus, Platypoecilus (now = Xiphophorus)[1] and other genera (Zolotnisky, 1901; Philippi, 1909; Seal, 1911; Henn, 1916; Christman, 1928; Stepánek, 1928; Ludwig, 1932; Collier, 1936; Schlosberg, Duncan and Daitch, 1949; Clark and Aronson, 1951; Clark and Kamrin, 1951; Clark, Aronson and Gordon, MS.; and many others).

In a number of families of fishes there is extensive morphological evidence as well as limited behavioral evidence for laterality (see review by Hubbs and Hubbs, 1945). The recent study of Turner (1950) on the morphology of the male gonopodium and female genitalia of Anableps substantiates in part Garman's (1895a; 1895b) idea that definite dextral and sinistral individuals of the two sexes exist and that copulation can occur only when fish of opposite laterality are paired. This means that dextral males can mate only with sinistral females and vice versa. In extensive studies of sexual behavior in the swordtail *Xiphophorus hellerii,* the platyfish *Xiphophorus maculatus,* and the guppy *Lebistes reticulatus* (Clark and Aronson, 1951; Clark, Aronson and Gordon, 1948; 1949; MS.) movements of the gonopodium to the right and to the left were recorded during swinging, thrusting and copulatory behavior. The present report offers an analysis of these data on the sideward movements of the gonopodium and a discussion of the general question of laterality in relation to ontogenetic and evolutionary processes. The problem of unilateral action of essentially bilateral structures has intrigued students of human behavior for many years, and much has been written on the subject. A few investigators have utilized the experimental approach in mammals, particularly in rats, but information concerning behavioral laterality in the lower vertebrates is particularly meager. It is hoped that through this study additional light may be shed on phylogenetic aspects of this problem.

METHODS AND RESULTS

The behavior of twelve male platyfish, *Xiphophorus* (*Platypoecilus*) *maculatus,* ten male swordtails, *Xiphophorus hellerii* and eight male guppies,

[1] In a recent publication, Gordon and Rosen (1951) reduced the genus Platypoecilus to the status of a subgenus and placed all the xiphophorin fishes in the genus Xiphophorus.

Lebistes reticulatus was analyzed in this study using the scores for right and left movements of the gonopodium during swinging, thrusting and copulatory actions. In swinging behavior the gonopodium is brought forward and to one side in conjunction with a forward movement of the homolateral pelvic fin. In thrusting behavior the forward and sideward movements are similar, but in addition, the gonopodium is directed toward the genital region of the female and may actually contact her genital orifice. Copulations are a prolonged variation of the contact thrust lasting as long as 5½ seconds during which sperm transfer is often effected.

The data were analyzed by utilizing the chi-square test as a means of estimating the probability that our samples represented a 1 : 1 ratio of rights and lefts. High values for chi-square would indicate that the right and left actions are not likely to be equally distributed. The scores are summarized in tables 1 to 3.

Swings. Column 5 in each of the following tables shows the individual chi-squares for swinging movements in each male. With one possible exception, none of the swing ratios in the three species studied deviates appreciably from the 1 : 1 hypothesis. The one doubtful case, platyfish male 4, scored 94 left swings to 68 rights, from which a chi-square of 4.17 is ob-

TABLE 1

RIGHT AND LEFT SCORES FOR SWINGS, THRUSTS AND COPULATIONS IN MALE PLATYFISH, *Xiphophorus* (*Platypoecilus*) *maculatus*

Male no.	Number of observations[1]	Swings			Thrusts			Copulations	
		Left	Right	χ^2	Left	Right	χ^2	Left	Right
1	16	44	30	2.65	147	116	5.78	5	7
2	16	63	50	1.50	142	94	9.76	12	12
3	16	98	82	1.42	352	204	39.40	1	0
4	16	94	68	4.17	125	198	16.50	1	1
5	16	63	69	0.27	120	161	5.98	1	1
6	16	90	79	0.72	287	117	71.53	14	6
7	17	99	118	1.66	62	233	99.12	1	10
8	7	24	25	0.00	11	38	14.88	0	4
9	17	114	119	0.11	280	192	16.41	0	0
10	17	112	97	1.08	148	316	60.83	1	1
11	17	107	99	0.31	47	137	44.02	0	0
12	17	81	92	0.70	303	469	35.69	0	1
Totals	188	989	928	14.59	2,024	2,275	419.90	36	43

ANALYSIS

	Swings			Thrusts		
	Degrees of freedom	χ^2	"P" values	Degrees of freedom	χ^2	"P" values
Total	12	14.59	0.27	12	419.90	< 0.01
Pooled	1	1.94	0.17	1	14.65	< 0.01
Interaction	11	12.65	0.31	11	305.25	< 0.01

[1]Duration of each observation was 10 minutes. Total observation time equals 3.1 hours.

TABLE 2

RIGHT AND LEFT SCORES FOR SWINGS, THRUSTS AND COPULATIONS IN *Xiphophorus hellerii*

Male no.	Number of observations[1]	Swings			Thrusts			Copulations	
		Left	Right	χ^2	Left	Right	χ^2	Left	Right
12	5	12	9	.43	58	35	5.69	1	1
14	5	6	8	.29	2	2	0.00		
15	5	14	15	.03	11	3	4.57		
16	5	13	10	.39	15	33	6.75		
17	5	21	27	.75	19	13	1.13		
18	5	5	5	.00	16	8	2.67	3	2
20	4	2	4	.67	1	2	0.34		
21	4	3	1	1.00	5	0	5.00		
22	5	4	6	.40	8	15	2.13		
23	5	5	3	.50	2	1	0.34		
Totals	48	85	88	4.46	137	112	28.62	4	3

ANALYSIS

	Swings			Thrusts		
	Degrees of freedom	χ^2	"P" values	Degrees of freedom	χ^2	"P" values
Total	10	4.46	.92	10	28.62	< 0.01
Pooled	1	.05	.83	1	2.51	0.12
Interaction	9	4.41	.88	9	26.11	< 0.01

[1]Duration of each observation was 10 minutes. Total observation time equals 8 hours.

tained. In samples of less than 200, Yates' correction is generally recommended to correct for the bias in associating the theoretical cumulative distribution of chi-square with the chi-square values obtained from enumeration (discontinuous) data. When this correction is applied in the case of platyfish male 4, the adjusted chi-square is 3.86 (P = .05), so that even in this case a significant departure from the 1 : 1 ratio is doubtful. The data were analyzed further by calculating the sum of chi-squares, the pooled chi-square and interaction chi-square as described by Snedecor (1946, p. 188). These additional procedures, summarized at the bottom of each table, do not indicate any significant deviation from the hypothesized ratio.

Thrusts (columns 6, 7 and 8 in tables 1 to 3). In the case of thrusts, however, the results do *not* conform to the 1 : 1 hypothesis in any of the platyfish males. Some males exhibited an excess of right thrusts—as high as 3.8 : 1 for male 7; others showed a predominant left tendency reaching 2.5 : 1 in male 6. Several of the swordtail and guppy males showed similar deviations. However, in the guppies, most of the scores were too small for critical analysis. A marked departure from the 1 : 1 ratio was indicated in all three species by the sum of chi-squares, the pooled chi-square and interaction chi-square. The high interaction chi-square obtained in each

case suggests that these groups were not entirely homogeneous and that the excess of rights in some of the males was neutralized by an excess of lefts in others. The pooled data therefore have limited meaning. This is particularly evident-in table 2 where a ratio of 137 : 112 thrusts may not be a significant departure from 1 : 1 (P = 0.12), but the high interaction chi-square of 26.11 (P = < .01) warns against too great a reliance on these pooled figures.

TABLE 3

RIGHT AND LEFT SCORES FOR SWINGS, THRUSTS AND COPULATIONS IN *Lebistes reticulatus*

Male no.	Number of observations[1]	Swings			Thrusts			Copulations	
		Left	Right	χ^2	Left	Right	χ^2	Left	Right
12	4	34	29	0.40	28	42	2.80	0	0
13	11	581	616	1.02	364	293	7.67	0	2
14	4	50	45	0.26	16	26	2.38	0	0
15	6	152	144	0.22	19	32	3.31	0	0
21	3	26	32	0.62	1	6	3.56	1	0
22	3	25	23	0.08	2	3	0.20	0	2
23	1	10	7	0.53	0	2	2.00	0	1
24	4	33	32	0.02	7	6	0.08	0	3
Totals	36	911	928	3.15	437	410	22.00	1	8

ANALYSIS

	Swings			Thrusts		
	Degrees of freedom	χ^2	"P" values	Degrees of freedom	χ^2	"P" values
Total	8	3.15	.92	8	22.00	< .01
Pooled	1	.16	.69	1	0.86	.37
Interaction	7	3.09	.87	7	21.37	< .01

[1]Observations of varying duration totaling 19 hours.

Thrusts, in contrast to swinging behavior, are always performed in relation to the female, hence the possibility exists that she may influence the direction of the thrusts. Since each test of a given male was performed with a different female, the results for individual tests made with each male were examined (when copulations did not occur, the females were occasionally used with other males). The scores for the 12 tests given platyfish male 1 are summarized and analyzed in table 4. Because the scores were low in most cases, adjusted chi-squares were used. Once more the data on swinging conform closely to the 1 : 1 ratio but several of the thrust scores significantly depart from this ratio. The sum of chi-squares, pooled chi-squares and interaction chi-squares for thrusts (based on unadjusted chi-squares) are all high. It can be seen clearly that in some tests there was a strong bias to the right; in others to the left. Hence, the pooled ratio of thrusts for male 1 used in table 1 does not present an entirely valid picture.

TABLE 4

RIGHT AND LEFT SCORES FOR SWINGS AND THRUSTS OF PLATYFISH MALE NO. 1

Observation date[1]	Swings			Thrusts			
	Left	Right	Adjusted χ^2	Left	Right	χ^2	Adjusted χ^2
10/20/49	3	2	0.0	7	1	4.5	3.13
10/21	1	0	0.0	4	0	4.0	2.25
10/22	6	6	0.0	1	5	2.58	1.50
10/25	5	2	0.57	4	4	0.0	0.0
11/1	3	3	0.0	1	1	0.0	0.0
11/5	8	5	0.31	7	0	7.0	5.3
12/8	0	1	0.0	10	23	5.12	4.36
12/9	3	3	0.0	34	14	8.33	7.50
12/13	0	0	....	22	14	1.78	1.36
12/14	0	0	....	1	2	0.03	0.0
12/15	3	2	0.0	14	13	0.04	0.0
12/21	2	1	0.0	3	13	6.25	5.06
12/22	1	1	0.0	13	6	2.58	1.89
12/23	5	3	0.13	26	20	0.78	0.54
12/29	0	0	....	0	0	....	....
1/3/50	4	1	0.80	0	0	....	....
Totals	44	30	1.81	147	116	42.99	

ANALYSIS

	Swings			Thrusts		
	Degrees of freedom	χ^2	"P" values	Degrees of freedom	χ^2	"P" values
Total	13	1.81	> .99	14	42.99	< 0.01
Pooled	1	1.41	.25	1	5.78	0.02
Interaction	12	.40	> .99	13	37.21	< 0.01

[1]Duration of each observation was 10 minutes.

Summaries of the analyses of the individual tests of the remaining 11 platyfish males for thrusts are presented in table 5. In all cases the sums of chi-squares are large and suggest marked departures from the 1:1 ratio, but in six males (Nos. 4, 6, 7, 8, 9 and 11) the interaction chi-squares are sufficiently small to indicate homogeneity. Hence in these cases the pooled ratios of thrusts in table 1 are more meaningful. Since these six cases all departed clearly from the 1:1 hypothesis, as the sum of chi-squares and pooled chi-squares shows, the indications are that these males exhibited a consistent and significant bias—two males sinistrally and four males dextrally—which was *independent* of the female used.

Although the data for the swordtail and guppy are not sufficient to warrant further statistical analysis, an inspection of these scores indicates a similar lateral tendency in certain males.

Copulation. Copulation scores are few, but sufficient to suggest strong laterality in certain males. In the platyfish, where these data are most numerous, there is some indication that the copulation bias correlates positively with the thrust bias. Thus the left-right thrust ratio for male 6 is

TABLE 5

ANALYSES OF CHI-SQUARES FOR RIGHT AND LEFT SCORES OF SWINGS AND THRUSTS IN THE TWELVE *Xiphophorus* (*Platypoecilus*) *maculatus* MALES

Male no.	Swings			Thrusts		
	Degrees of freedom	χ^2	"P" values	Degrees of freedom	χ^2	"P" values
1 Total	13	1.81	> .99	14	42.99	< 0.01
Pooled	1	1.41	.25	1	5.78	0.02
Interaction	12	.40	> .99	13	37.21	< 0.01
2 Total	16	3.78	> .99	14	34.25	< 0.01
Pooled	1	1.26	.27	1	9.76	< 0.01
Interaction	15	2.52	> .99	13	24.49	0.03
3 Total	16	3.65	> .99	11	82.55	< 0.01
Pooled	1	1.25	.26	1	39.40	< 0.01
Interaction	15	2.40	> .99	10	43.15	< 0.01
4 Total	16	4.49	> .99	14	30.17	< 0.01
Pooled	1	1.94	.18	1	16.50	< 0.01
Interaction	15	2.55	> .99	13	13.67	0.40
5 Total	15	3.01	> .99	12	33.54	< 0.01
Pooled	1	.18	.68	1	5.98	0.02
Interaction	14	2.83	> .99	11	27.56	< 0.01
6 Total	15	.85	> .99	15	91.80	< 0.01
Pooled	1	.59	.46	1	71.53	< 0.01
Interaction	14	.26	> .99	14	20.27	0.13
7 Total	17	3.11	> .99	15	116.29	< 0.01
Pooled	1	1.50	.23	1	99.12	< 0.01
Interaction	16	1.61	> .99	14	17.17	0.25
8 Total	6	.13	> .99	5	17.29	< 0.01
Pooled	1	0.0	.99	1	14.88	< 0.01
Interaction	5	.13	> .99	4	2.41	0.66
9 Total	17	1.77	> .99	14	29.76	< 0.01
Pooled	1	.04	.76	1	16.41	< 0.01
Interaction	16	1.73	> .99	13	13.35	0.42
10 Total	17	3.79	> .99	16	85.61	< 0.01
Pooled	1	.98	.42	1	60.83	< 0.01
Interaction	16	2.81	> .99	15	24.78	0.05
11 Total	17	5.30	> .99	12	60.16	< 0.01
Pooled	1	.24	.64	1	44.02	< 0.01
Interaction	16	5.06	> .99	11	16.14	0.14
12 Total	16	3.26	> .99	13	72.44	< 0.01
Pooled	1	.58	.46	1	35.69	< 0.01
Interaction	15	2.68	> .99	12	36.75	< 0.01

287 : 117 and the copulation ratio 14 : 6. Similarly, the thrust ratio for male 7 is 62 : 233 and that for copulation is 1 : 10.

DISCUSSION

Observations of courtship behavior show that the males of all three species tend to alternate gonopodial swings; first to one side, then to the other. This is substantiated by a statistical analysis of scores for swinging which

reveals a very low probability that any further samples would have deviated further from a 1 : 1 ratio. In contrast to this, the thrust and copulation scores of many of the males are clearly biased—some to the right and others to the left. Moreover, the full effect of the right or left tendency is probably masked in several cases (e.g., platyfish Nos. 1, 3, 5, and 12) by the female acting as an independent factor in determining the direction of the gonopodial action. Such an influence seems to be exerted through the position taken by the female in relation to the walls of the aquarium. Frequently it was noted that the female remained stationary close to a wall while permitting the male to thrust or copulate. Hence the thrusts and copulations were of necessity from only one side, so that any laterality in the male would be either reinforced or neutralized. Thus, swinging is found to be an ambidextrous activity in all the individual males of the three species analyzed, whereas in thrusting and copulatory behavior individual tendencies for right or left biases clearly exist.

It should be stressed, however, that this laterality is not absolute in any of the males tested. All males were capable of thrusting to both the left and right sides and the data show to a limited extent that individual males are also capable of copulating and inseminating females on either side.

These data were restricted by the fact that during most observations two or more copulations took place and these were often to opposite sides. Hence sperm smears taken at the end of the observations did not reveal on which side (or sides) insemination was accomplished. However, records on three males show that they copulated and inseminated on the left side in some observations and on the right side in others.

Additional data collected in connection with a more extensive study support our contention that an individual male can copulate and inseminate on both sides. This is in strong contrast to the fixed sexual dextrality or sinistrality found in individual males of Anableps, the phallostethids, as well as in members of the Indo-Pacific viviparous halfbeaks referred to the family Dermogenyidae (Hubbs and Hubbs, 1945) where in all probability the strongly asymmetrical genital morphology specifically restricts copulation to one side only. Unfortunately, data based on direct observation of mating behavior in these groups is limited.

An examination of gonopodial morphology in males of certain xiphophorin species (including *X. maculatus* and *X. hellerii*), led Hubbs and Hubbs (1945) to conclude that there exists a "fixation of the concavity [transitory groove] on one side of the gonopodium in the Poeciliidae." These authors reported that "as many males have the gonopodium concave on the left side as on the right." This is suggested as evidence for an asymmetry in reproductive behavior. This conclusion derived from a study of preserved material should now be revised in the light of our behavioral studies. Actually, the equal number of right and left males indicates a 1 : 1 probability that an individual male will be fixed with the transitory groove to either the right or the left side. If the gonopodium is examined while a living individual is lying on the plate of a dissecting microscope the transitory groove can

be seen to shift from one side to the other depending on slight movements of the fish. Since the gonopodium cannot be pulled directly forward without placing abnormal stress on the fin tissues, it shifts to either the right or left side when forward pressure is exerted by the experimenter. A transitory groove then forms on the corresponding side. It is not known, however, whether a positive correlation exists between the left or right thrust tendencies in individual platyfish males and the position that their gonopodia would assume on preservation.

The movements of the gonopodium during copulation in the guppy, platyfish and swordtail are discussed by Clark and Aronson (1951) and Clark, Aronson and Gordon (MS) and are analyzed in detail in xiphophorins by Rosen and Gordon (MS). In general, statements in these publications concerning the bilateral nature of gonopodial movements and transitory groove formation are supported by the statistical data presented in this paper.

Bearing in mind that swinging, thrusting and copulation are rather similar actions, the question now arises why swinging behavior is approximately symmetrical while in the same individuals thrusting and copulatory behavior may be noticeably asymmetrical. An adequate answer to this question is not forthcoming but two suggestions are offered. First, during thrusts and copulations the gonopodium is directed toward the genital region of the actively moving female. Thus the action required is more precise than in swings. It is at these times that differences in the neuromuscular mechanisms on the two sides might be effective. Secondly, the high level of nervous excitability associated with thrusting and copulatory behavior might bring out asymmetrical actions not apparent during the less stimulative swinging behavior.

With good cause, Hubbs and Hubbs (1945) are disturbed by the widespread belief that handedness is a phenomenon peculiar to man, or that handedness in man is not related to observed bilateral asymmetries in other animals. However, these investigators base their argument primarily on widespread morphological asymmetries found in fishes; in other words, evidence concerning structural laterality in fishes is compared with evidence for laterality in man which is largely behavioral. The few behavioral asymmetries in fishes for which Hubbs and Hubbs have evidence are correlated with gross structural differences between the two sides which limit the possibility of ambidextral behavior. In the platyfish and swordtail where the gonopodium is structurally symmetrical, we have demonstrated a slight but statistically significant dextrality and sinistrality in the thrusting behavior of some platyfish individuals and would anticipate the same condition in the swordtails. However, in comparison with handedness in man or handedness in rats, where certain acts are performed mostly or exclusively with one or the other hand, these fishes would be characterized as ambidextrous. It should be observed that while the gonopodium is a median structure, the neuromuscular mechanism associated with it is bilaterally arranged, so that comparisons with more typically bilateral structures seem justified.

As pointed out by Hubbs and Hubbs, more attention should be given to the biological aspects of handedness in man, and factors common to all asymmetries should be sought. Such factors are suggested by studies on handedness in chimpanzees (Finch, 1941), monkeys (Coles and Glees, 1951), horses (Grzimek, 1949), rats (Yoshioka, 1930, 1930a; Tsai and Maurer, 1930; Peterson, 1934; Peterson and Fracarol, 1938) and parrots (Friedmann and Davis, 1938; Grzimek, 1949). However, a very cautious attitude is necessary in comparing morphological asymmetries in fishes with lateral physiological dominance of the essentially symmetrical mammalian structures dealt with in the studies mentioned above. In view of the very fundamental differences between the neural organization of fish and man, the relationship between behavioral laterality in fish and handedness in man may be rather remote. Consequently, Hubbs and Hubbs may go too far when they say in their summarizing statement that, "The frequent occurrence of bilateral differences between the forelimbs and between left and right elements of many other structures of fishes counters the widespread idea that handedness is a peculiarly human trait, or the result of training."

Breder (1947, p. 357) has pointed to the selective advantage of first degree right-left reflective symmetry (bilateral, mirror image symmetry in ordinary usage) to locomotion. Thus, according to Breder, on purely functional grounds there would seem to be little reason why a sessile organism such as an oyster should be limited to building itself into a particularly restricted symmetrical body. "On the other hand if mobility is of value, symmetry of the first degree must be invoked because of streamline necessity."

Yet, the frequency of evolutionary trends away from this condition suggests advantages in asymmetry, although to be sure these are not always obvious. Occasional appearances of asymmetry may be regarded as reactions to specific environmental situations, the resulting configurations representing a balance between the disadvantages of asymmetry to locomotion and whatever advantages the particular deviation may afford. Since ontogenetic processes have a tendency to form symmetrical structures (Breder, 1947) we cannot think of species lapsing into asymmetry whenever the restrictive forces of natural selection are relaxed. Rather we should attach appropriate weight to whatever specific situations (both environmental and genetic) may have led particular species away from structural and functional symmetry.

SUMMARY AND CONCLUSIONS

Swings, thrusts and copulations to both right and left were recorded during several series of mating observations on twelve male platyfish, ten male swordtails, and eight male guppies. These data were analyzed by means of the chi-square technique as a test of conformity to a 1:1 hypothesis of rights and lefts. Swing scores in all three species conformed closely to this hypothesis, indicating that in the performance of this behavior there is no demonstrable asymmetry of function. Thrusts which are directed towards the female showed small but statistically significant deviations either to

the right or left in all species tested. Further analysis of the data in the platyfish indicated that some males exhibited small but significant biases in thrusting behavior to either the right or left. These biases were independent of the females with which the males were paired. Copulation scores were too limited for adequate statistical analysis, but right or left tendencies in this behavior are suggested. It is probable that males can copulate and inseminate in either direction. While some males showed slight preferences for one side or the other during thrusting behavior, this laterality does not compare too closely with handedness in mammals and humans where specific procedures are conducted almost exclusively with one hand.

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THE GRAFTING RELATIONS OF WILTY DWARF, A NEW TOMATO MUTANT

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Striking formative effects of stock upon scion and of scion upon stock in grafts of higher plants are nothing new to the horticulturist. The dwarfing influence that certain stocks exert upon scions of fruit trees and woody ornamentals, for instance, has long been exploited by pomologists and nurserymen. In the light of present knowledge of translocated growth-promoting substances and other intricate relations between shoot and root systems of higher plants it would seem that the absence should attract more interest than the presence of these stock-scion modifications. Physiological and other aspects of this subject have been reviewed extensively by Roberts (1949).

The best known examples of stock-scion modifications, being woody and highly heterozygous, prove to be the poorest materials for a test of the hereditary transmission of such effects. Annual or perennial dicotyledonous herbs are much better adapted and have been utilized almost exclusively for such tests. The tomato (*Lycopersicon esculentum* Mill.) is a favorite subject primarily because it is easily propagated asexually both by grafts and by rooted cuttings, its flowers are automatically self-pollinated yet readily allow controlled matings, and it offers a wealth of pure-breeding varieties and sharply distinguished mutants.

The literature reveals a strong disagreement between reports, on one hand, chiefly by Soviet workers, of marked immediate and hereditarily transmitted effects, and, on the other hand, of those of no immediate or transmitted effects or of immediate effects not transmitted to seminal offspring. A complete review of the literature is not attempted here, but a few representative reports can be mentioned.

As typical reports of positive alterations of the germ plasm, Gluščenko (1946, 1947, 1950) describes changes induced in the locule number, color, and size of fruits of scions and stocks, but especially of the latter, in graft combinations of different tomato varieties and species. In most cases the characters were modified to resemble those of the other symbiont, but in at least one instance, an entirely new character appeared. The modifications also appeared in the first and second seminal generations. In grafts of tomato on nightshade, tomato on belladonna, and reciprocals, alkaloid content was increased in the tomato symbiont and its sexual offspring (Braslavskaja, 1948). Haschkova (1944) reports that in grafts of tomatoes and eggplant fruit weight was increased and seed number greatly decreased in the tomato symbionts and their progeny. According to Aizenshtat (1949) dominance of scions can be altered by recessives used as stocks. Flowers of the scions possessing the dominant alleles

C and *R* were crossed with the corresponding recessives. Recessive traits were expressed to some extent in the F_1 and the proportion of recessive offspring in the F_2 greatly exceeded the normal expectation.

Reports of negative findings are less abundant apparently because investigators are no more likely to be attracted to experiments that by the implied contradiction of well established modern concepts of genetics are likely to yield negative results, than they are to the reporting of negative results. As to the immediate effects induced by grafting tomatoes, Wilson and Withner (1946) found no changes in visible morphology and no alteration in content of certain major B vitamins in symbionts despite large differences between stock and scion. Likewise, Walker (1951) found no modification in the phenotype of tomatoes grafted reciprocally with *Cyphomandra betacea* Sendt. Howard (1944) reports negative findings in grafting experiments with peas and later (1949) with potatoes, and in the latter publication refers to unpublished negative findings of Hawkes on potatoes and of Bates on tomatoes.

In the present experiment phenotypes of the grafted symbionts were greatly affected, but no hereditary transmission of such changes was detected. Also, certain immediate changes induced by grafting were not found in the progeny in the experiments of Glušč enko (1946, 1947). Current Soviet concepts concerning environmental modifications of the germ plasm must be extremely elastic to explain such findings.

Exploratory tests here have revealed no immediate effect on scions of reciprocal grafts of lines homozygous for the genes a_1, *c*, d_1, *f*, *H*, *j*, *lf*, ms_3, *r*, *Wo*, *wt*, and *y* in combination with lines of the normal alleles (Plaisted, Rick, unpub.). Strong modifications were found, however, in graft combinations of a new mutant, the subject of the present study. Further investigation of the grafting relations of this mutant was also prompted by the aforementioned controversy in the literature concerning graft-induced modifications of the germ plasm.

DESCRIPTION OF THE MUTANT

The mutant "wilty-dwarf" (No. 2-110) used in these experiments was discovered in a commercial field of the tomato variety San Marzano during the course of a search for male-sterile mutants. It was distinguished from surrounding normal plants, not so much by unfruitfulness and consequent great vegetative growth characteristic of sterile mutants, as by its peculiar dwarf erect habit and unique color of foliage. Although not so freely fruitful in the field as normal tomatoes, it sets seedy fruits readily after artificial self-pollination. Its generative organs do not display any marked modifications that might prevent normal self-pollination and might thereby explain the unfruitfulness.

Wilty dwarf differs from normal tomatoes in many vegetative characters. Total growth of the plant is so greatly limited that by the end of the season mutant plants are less than one-tenth as large as normal plants, the difference reflecting a reduction in the length and number of internodes, espe-

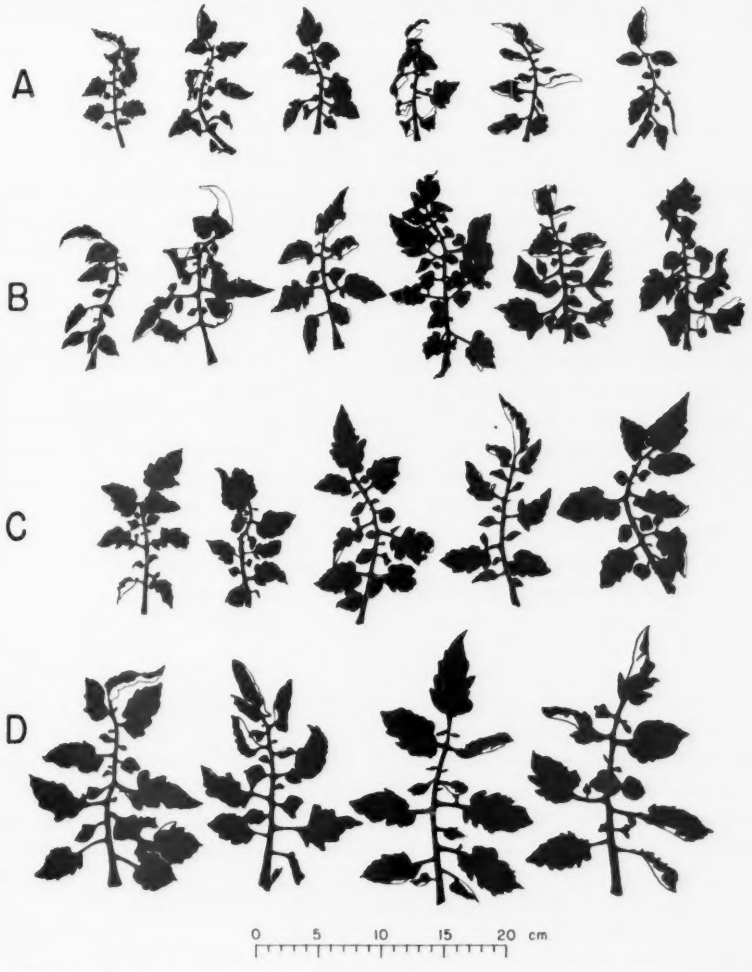


FIGURE 1. Leaf outlines of typical scions of grafts of wilty dwarf (*wd*) and normal type (+). In each group the topmost mature leaves of a single stem are placed in order consecutively according to age, the youngest on the left. FIGURE 1A—leaves of scion of *wd/wd*. FIGURE 1B—leaves of scion of *wd/+*. FIGURE 1C—leaves of scion of *+/wd*. FIGURE 1D—leaves of scion of +/+.

cially the latter. Stem girth and size of leaf blade (see fig. 1A and 1D) are also greatly reduced. During the day in summer the leaves droop as if the plant suffered from lack of water, but no matter how much water is supplied by irrigation, the leaves do not regain normal turgor. The mutant, in fact, wilts much more realistically than *wt*, a previously described (MacArthur, 1934) mutant, in which leaves roll at their edges but remain turgid. Another feature of the mutant is a distinct grayish-blue cast of

the leaf. The aforementioned traits are much less manifest under greenhouse conditions, where, save for slight differences in leaf color and internode length, the segregants are difficult to identify.

The mutant condition was demonstrated to be heritable and the original plant to be homozygous by the fact that all of its progeny, consisting of 47 plants in five families, were wilty dwarf. All individuals in many F_1 families derived from crosses between wilty dwarf and normal have been normal. Up to the present time nine F_2 families have been raised, the total count in which is 341 normal and 125 wilty dwarf. The deviation from 3:1 is 8.5; the Chi-squares corresponding to this deviation and also to heterogeneity are not significant. The mutant character therefore is probably determined by a single recessive gene, to which the symbol *wd* is applied.

All of the mutant features enumerated above appear in each of the *wd* segregants as if all were determined by the same gene.

The line 2-72 of San Marzano (hereafter designated as +) was used as the normal type for grafts with *wd*. The former, having been derived by doubling the chromosome number of a haploid by means of colchicine, is a typical line of the variety, and, as might be anticipated from its mode of origin, is a very uniform line. The two lines differ, therefore, in a single major gene, and not, so far as the morphology of parents or their hybrid progeny reveal, in any other respect.

METHODS

Stock and scion were united by means of the cleft graft, the base of the scion being cut in the form of a wedge, which was forced into a vertical slit cut in the decapitated stem of the stock. Age of the tissue cut did not seem to affect success of the graft, although all plants were relatively young, having only produced their first flowers at the time of the operation. As a precaution to prevent loss of moisture, the grafted parts were wrapped with a narrow strip of pliofilm, which, in turn, was coated with melted paraffin heated only sufficiently to prevent it from solidifying. The scion was temporarily covered with an inverted glass vial, which was closed with a plug of moistened cotton to maintain high humidity.

At the first evidence of growth of the scion, the vial was removed, and the region of the graft permanently marked. Careful attention was required thereafter for the removal of all but one of the lateral shoots of the stock. With this method 75 per cent or more of the grafts succeeded.

Mutant and normal plants were grafted reciprocally, and control grafts of *wd* on *wd* and of + on + were made. Grafts of these combinations and also ungrafted plants were planted two feet apart in random order in a single row in the field. All donors of scions were grown in the field as an absolute check on the phenotype of scions. By appropriate pruning, growth of the scion and a single lateral shoot of the stock was limited to single stems, which were trained to a stake.

For the sake of convenience any graft combination will be designated hereafter as a fraction, the numerator referring to the scion, the denominator

to the stock. Since all lines grown proved to be homozygous, a single symbol is sufficient for either type. As an example, *wd*/+ indicates a graft combination in which the scion is wilty dwarf and the stock is normal.

IMMEDIATE EFFECTS IN SINGLE GRAFTS

The term "immediate effects" is intended to refer to effects on the scion or stock during the growth of the graft combination in contrast to any effect that might appear in the progeny of grafted plants.

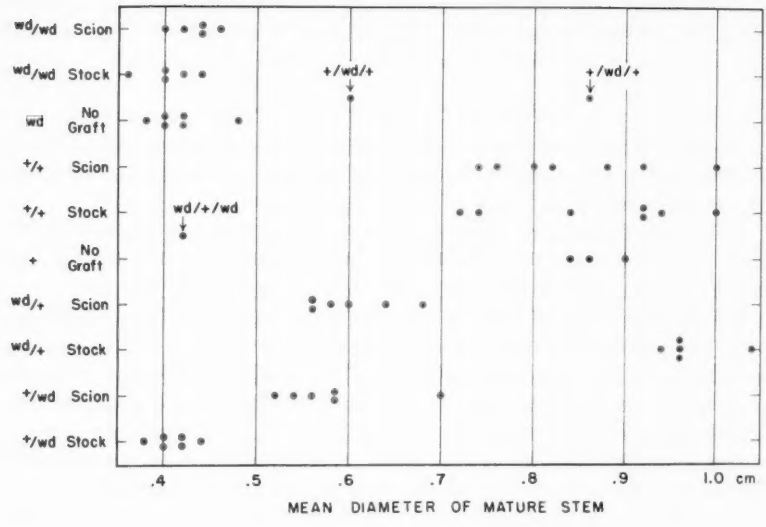
A few plants of each possible graft combination were planted in the field in 1950. Before the plants had grown much, a modification of the phenotype of scions of the *wd*/+ and +/*wd* grafts became apparent. Size of leaf and thickness of stem of *wd* scions of *wd*/+ were greatly increased, while the same dimensions of + scions of +/*wd* were greatly decreased, revealing that the stocks tended to alter the scions to resemble the stock in reciprocal grafts of *wd* and +. This modification was so great, in fact, that in respect to these two aspects, scions of *wd*/+ resembled ungrafted + more than the scions of +/*wd*. Other phenotypic differences between *wd* and + such as number and length of internodes, wiltiness and color of leaves appeared to be autonomous in scions of heterogeneous grafts, being modified little if any by the stock.

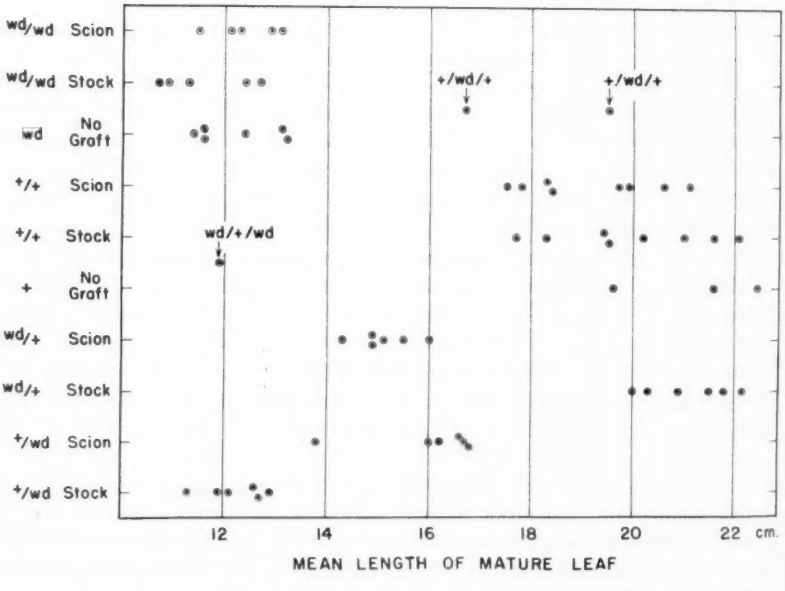
Scions of the control grafts, *wd*/*wd* and +/+ closely resembled ungrafted plants of the same genotype; the graft operation itself could not therefore account for any of the changes observed in the heterogeneous grafts.

In 1951 all grafts were repeated on a somewhat larger scale, five to seven plants of each graft combination surviving to the end of the growing season. The plants were handled in the same fashion as those grown in 1950. The lines of + and *wd* were demonstrated to be genetically pure by the fact that no segregation occurred in either the group used as stocks or the donor plants from which scions had been taken.

On the basis of the experience of 1950, measurements were made in 1951 of the leaves and stems of the test plants. Length of leaf from base of petiole to tip of blade was adopted as the measure of leaf size, and girth of stem was assessed by measuring stem diameter. The figure taken as representative of a plant is the mean of lengths of all mature leaves developed after an arbitrary date and the mean of five separate measurements of stem diameter in successive internodes below the third leaf of mature size.

The distributions of stem diameter and leaf length for the various graft combinations and ungrafted plants are presented graphically in figures 2 and 3. In the main, the new figures bear out the observations made in 1950. The dimensions are so greatly modified in scions of heterogeneous grafts that no statistical calculation is needed to demonstrate the significance of differences between them and controls; the distribution of the former, in fact, do not even overlap those of the + or *wd* controls. The mean stem diameter for scions of *wd*/+, 0.60 cm. is slightly greater than the 0.58 recorded for scions of +/*wd*, control means for scions of *wd*/*wd*





FIGURES 2 and 3. Distributions of mean measurements of wilty dwarf (*wd*) and normal type (+) and of scions and stocks in various graft combinations of *wd* and +. Each dot represents the mean of measurements for one plant. FIGURE 2 (above)—distributions of stem diameter. FIGURE 3 (below)—distributions of leaf length.

and +/+ being respectively 0.43 and 0.85. The situation in leaf lengths is similar, though the shift is not quite so great (see fig. 3). Here the mean length of leaf for scions of *wd*/+ is 15.1 cm., of +/*wd*, 16.0, of *wd*/*wd*, 12.4, and of +/+, 19.3. As in 1950, the grafting operation, *per se* seemed to have no effects on scion development. Thus, although the leaf lengths of scions were not shifted quite so markedly in 1951 as in 1950, stocks affected development of the scions in the same direction and to a degree that was obvious despite other influential factors of the environment.

Striking as these changes are in linear dimension, they do not give the impression of the extensive modification of the organs in two dimensions. In order to give a clearer impression of the immediate effects of stock on scion, outlines of leaves from scions of typical reciprocal and control grafts of *wd* and + are presented in fig. 1.

Turning attention now to stocks, we see a possible influence of scion upon stock in the *wd*/+ combination, but the effect, if one really exists, is in the reverse direction. Thus, while leaf and stem dimensions are diminished in scions of +/*wd*, they are augmented in stocks of *wd*/+ (see figs. 2 and 3). Slight, if any, evidence exists for a reverse influence of the same or any sort on stocks of +/*wd*. Although the meaning of this reverse effect of scion on stock is not clear, the similarity in leaf length of ungrafted plants of + and of stocks of *wd*/+ suggests the following explanation. Vigor of the shoot and, by the same token, dimensions of its organs seem to diminish as the number of shoots increases. Now the growth of *wd* is so weak that the + stocks of *wd*/+ might behave more like a plant with one shoot (+, no graft in fig. 3) than plants with two shoots (scion and stock of +/+).

IMMEDIATE EFFECTS IN DOUBLE GRAFTS

In both 1950 and 1951 it was possible to secure double grafts of the +/*wd*/+ combination. The second graft was made three or four internodes above the first as soon as the scion of the latter had resumed vigorous growth. In both years the second grafts had to be made in hot weather unfavorable for grafting, and only one double graft of this type survived in each year. Although only two plants were available, the measurements are remarkably consistent and indicate interesting relations. In both instances, dimensions of the shoot from the *wd* scion were markedly modified as they are in the *wd*/+ combination. But the + scion at the top displayed typically + dimensions (figs. 2 and 3) thus apparently not being affected by the scion of *wd* intercalated between it and the + stock. On the basis of this meagre but consistent evidence, the effect of the *wd* stock upon the + scion seems to be vested in an influence not from the stems or leaves of the stock but from its root system.

One specimen of the reciprocal double graft, *wd*/+/*wd* was produced. No shoot developed from the + scion but measurements place the *wd* top scion well within the range of *wd* and therefore suggest again that the strong effect of stock upon scion emanates from the root system and not from hormones or any other physiological effect of the stem or leaf.

The intrinsic nature of this influence of stock upon scion remains to be explained. Superficially it seems quite possible that the observed results could be explained by some simple defect in the vascular system of the *wd* root system that might prevent adequate transfer of water to the shoots of either *wd* or normal above it. According to this proposal + root systems would maintain normal growth of + and enhance the growth of *wd* shoots. On the other hand, some more subtle influence such as production of a growth-regulating substance by the root system might be responsible. Dawson's work (1942) on grafts of tobacco and tomato is significant in this connection. By the presence of nicotine in tomato scions grafted on tobacco stocks and its absence in scions of the reciprocal combination he demonstrated that nicotine or a precursor is synthesized only in the roots of tobacco and is translocated to the shoots. Although nicotine apparently does not influence growth of the scion, this pattern of generation and translocation might also apply to substances having formative effects. A difference in selective absorption by membranes of the root system might also account for the results of the present experiment. Whatever the explanation, it cannot account completely for the phenotype of *wd*, for, as already explained, internode dimensions and color and wilting of the leaf remain largely autonomous in scions whatever the genotype of the stock.

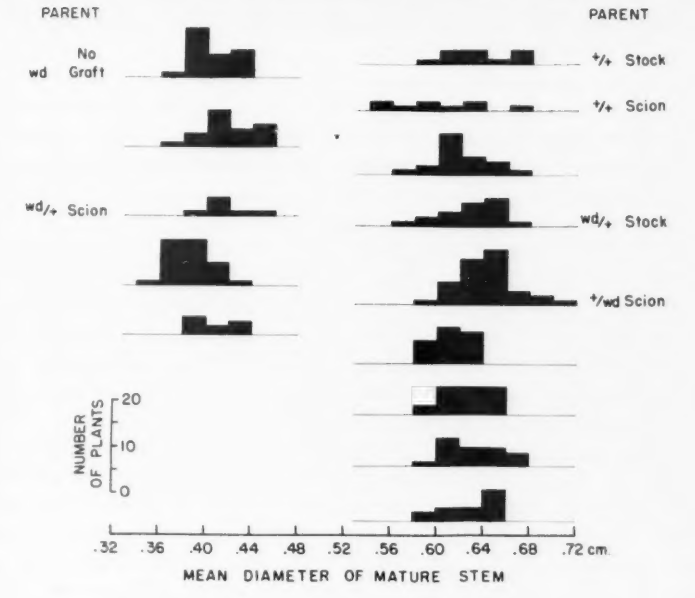
These grafting experiments reveal that stocks strongly modify the growth of scions in the combinations *wd*/+ and +/*wd* and that the influence of the stock is vested not in its stems or leaves but in its root system.

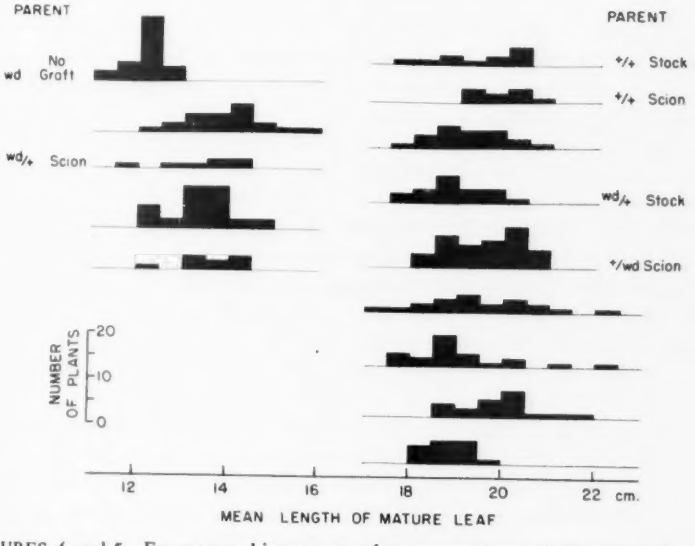
CHARACTERISTICS OF OFFSPRING OF GRAFTED PLANTS

Certain flowers of scions and stocks of some of the grafted plants of 1950 were artificially self-pollinated. Seeds were saved and planted in 1951 for observation of the next generation. Small families were grown, each being derived from a single fruit of a different parent scion or shoot. There being no apparent need for pruning and staking as for the grafted plants, individuals of the progenies were left to grow on the ground without alteration.

In studying these families it became obvious immediately that all plants in the progenies derived from + scions or + stocks were all of + phenotype and all derived from *wd* scions were of *wd* phenotype regardless of the genotype of the other symbiont in the graft combination. Nothing that was observed could be interpreted as an intermediate type in any way simulating the modification observed in scions of the preceding generation. On the basis of all the character differences manifest in *wd*, each plant obviously fell qualitatively in either + or *wd* phenotypes. In short, no graft influence on qualitative differences was observed in the offspring.

Besides these general observations, the stem and leaf characters were measured for each plant in the same fashion as those of the parent plants in order to make quantitative comparisons with the latter. Distributions of the recorded plant means are represented in the form of bar histograms in figs. 4 and 5. Here again it is evident that the graft mate of any particular





FIGURES 4 and 5. Frequency histograms of measurements of the progenies of grafted plants of wilty dwarf (*wd*) and normal type (+). Parentages are indicated in the columns to the right and left of the distributions. FIGURE 4 (above)—distributions of stem diameter. FIGURE 5 (below)—distributions of leaf length.

combination does not affect the quantitative characters of the progeny any more than it does the qualitative features already described. The populations fluctuate appreciably in respect to means and variability, but these differences do not correspond to any parental graft combination and likely represent differences in environmental conditions in the field. The large hiatus between + and *wd* is maintained for stem diameter (fig. 4) and leaf length (fig. 5) just as it was in the parent control grafts and ungrafted plants. In terms of absolute measure, mean leaf lengths of all of the + progenies are somewhat lower than those of the parent plants, and the distributions of stem diameter of the + progeny fall completely below those of the parent plants, regardless of the graft combination. This difference is interpreted as an immediate environmental effect most likely explained by the greater vigor of the branches of the grafted plant pruned to one or two stems in contrast to the reduced vigor of the many branches of the offspring, which were not pruned in any fashion.

For exact quantitative comparisons of the progeny families, means were calculated for each distribution and then averages of means for distributions of similar parentage were calculated and are listed as follows: (leaf length) scion of *wd*/+, 13.44 cm.; scion of +/wd, 19.45; *wd* control, 13.02; + control, 19.33; (stem diameter) scion of *wd*/+, 0.404 cm.; scion of +/*wd*, 0.629; *wd* control, 0.411; + control, 0.625. In three of the four comparisons progeny means of grafted plants thus deviate from corresponding controls in the opposite direction from that of the parent scion; in other words, they are shifted in the direction opposite to that expected if the graft-induced changes in the scion were inherited. The differences are all small and doubtlessly not significant. In the remaining case, mean leaf length of progeny of scions of *wd*/+ exceeds that of the *wd* control thus constituting the only apparent shift of progeny expression toward the value of the stock. But inspection of the two control curves (fig. 5) reveals that they are greatly separated and that the range of the control curve of higher values includes the levels of any of the three treatment curves. Even in this instance it therefore appears very doubtful whether the stock induced any change in the scion that was transmitted to the progeny.

In conclusion it can be stated that although the experiment demonstrates a marked immediate effect of stock on scion, it does not reveal any effect of similar nature and magnitude transmitted to the next generation. If any such effects are inherited, they must be of such very small magnitude that a replicated test conducted on a very large scale would be needed to reveal them.

Although the present experiment was conducted under different conditions and with different genetic materials than those on which reports of positive graft-induced alterations of the germ plasm are based, it is doubtful whether such differences could account for the contradictory results. It also seems likely that if any alteration in scion morphology could be transmitted to seminal offspring, such transmission would be expected in the present ex-

ample, in which the alterations are so profound. In the light of this experience it seems doubtful that the conflicting reports can be reconciled.

After conducting such an experiment one becomes aware that a false impression of hereditary changes might be conveyed by a mistake in any of several steps. Chance outcrossing of parental plants, for instance, would provide stocks for an experiment that could lead to completely erroneous conclusions as could also accidental mixing of scions, failure to mark the position of grafts, contamination of the selfings or controlled mating of symbionts, and mistakes in pedigreeing the offspring. On the other hand, it is extremely unlikely that errors would consistently lead to negative results. Variable and inconsistent results purported to demonstrate vegetative hybridization should therefore always arouse suspicion. It also follows that graft hybridization cannot be accepted as a reality until consistently demonstrated by many experiments that are conducted very carefully and independently by disinterested investigators.

SUMMARY

Wilty dwarf is a new tomato character that is determined by a single recessive gene, *wd*. It differs from normal (+) in the following features: erect dwarf habit (fewer and shorter internodes), smaller leaves, thinner stems, blue-gray leaf color, and tendency of foliage to droop under field conditions in summer. Although fruitfulness is somewhat subnormal, flowers will set fruits with abundant seeds if artificially self-pollinated. The normal stock used for grafting is a pure line derived from a haploid of the same variety and apparently differs from the mutant only in having the + allele of *wd*.

Homozygous lines of *wd* and + were grafted in all possible combinations. Whereas the control grafts *wd*/*wd* and +/+ retained their phenotype without modification, scion stem diameter and leaf length of the reciprocal grafts of *wd* and + were shifted markedly toward the dimensions of the stocks. These modifications were so great that stems and leaves of scions of *wd*/+ had approximately the same dimensions as those of +/*wd*, their frequency distributions not even overlapping those of the *wd* and + controls. In respect of other character differences between *wd* and + the scions remained essentially autonomous. The data also suggest a possible effect of scion on stock, but of a reverse direction and smaller magnitude than that of stock on scion.

In a limited number of the reciprocal double graft combinations *wd*/+/*wd* and +/*wd*/+, the top scion in all specimens showed characters of the donor without modification, whereas the middle scion was altered in the same fashion as in the single grafts. It is evident, therefore, that the effects of stocks upon scions, whatever their intrinsic nature, are generated not by the stems or leaves, but by the root system of the stock.

Progeny from selfed flowers of the grafted plants were grown and measured in the same fashion as the parent plants. All plants in the progenies

corresponded to the genotype of the parent tissue in respect to the qualitative and quantitative differences between *wd* and + regardless of the parental graft combination. Thus, in spite of strong immediate effects of stocks on scions, the experiment did not reveal transmission of such effects to the seminal progeny.

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INHERITANCE OF VARIATIONS IN THE MALE GENITALIA IN DROSOPHILA PAULISTORUM

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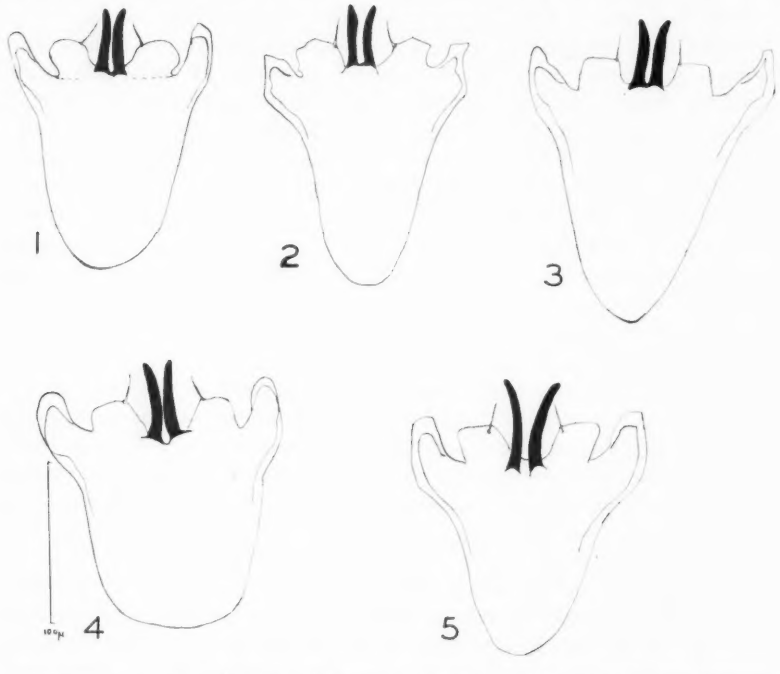
It has been known for more than a century, since the work of Dufour (1844), that species of insects usually differ in the morphology of their genitalic armatures. Clear-cut differences in the structure of the genitalia are often found even between closely related species which are difficult to classify by their external characteristics. This fact served as the basis of Dufour's "lock-and-key" theory, according to which insect species are maintained owing to a close correspondence of the male and female genitalia, while distinct species are kept apart by non-correspondence of their genitalia. Modern work has shown that mechanical isolation is less important than was thought by the older authors (see Dobzhansky, 1951). Nevertheless, since very little is known about genetics of isolating mechanisms, information regarding the genetic causation of variations in the genitalia is greatly to be desired. Shull (1946) appears to be the only investigator so far who has submitted this problem to experimental study. He found that the differences between the male genitalia of two species of ladybird beetles, *Hippodamia quinquestignata* and *H. convergens*, are determined by interaction of many genes with individually small effects (polygenes). The purpose of the present note is to report some observations on differences in the structure of the male genitalia found between certain populations of *Drosophila paulistorum* Dobzhansky and Pavan. In contrast to Shull's findings, the intraspecific variations observed in Drosophila proved to be genetically simple.

Drosophila paulistorum is a species distributed from the state of Rio Grande do Sul, in southern Brazil, to the West Indies. As shown by Burla et al. (1949), this species is particularly common in the wet equatorial jungles of Brazil; towards the margins of its distribution area it becomes relatively rare compared to a related form, *D. willistoni* Sturtevant. The northernmost known localities of *D. paulistorum* are the Isle of Trinidad (collected by Dr. C. P. Haskins) and Soledad, Cuba (collected by Mr. J. I. Townsend, who generously permits the use of his unpublished findings).

The genitalia were examined in three strains derived from single females collected at Osorio, in the state of Rio Grande do Sul. The hypandria (Malogolowkin, 1951) in these strains resemble that shown in figure 1. Their characteristic features are rounded paired lobes on the margin, each lobe bearing a single bristle. In the strains from the state of São Paulo (fig. 3) these lobes are distinctly angular in shape. Several strains were examined, each derived from several females caught at Mogi das Cruzes and kindly placed at the author's disposal by Professor C. Pavan. The same angular shape of the lobes appears in the drawing of the hypand-

rium of *D. paulistorum* published by Burla et al. (1949), whose material was also derived from Mogi das Cruzes. The males from the strain of the same species collected by Th. Dobzhansky at Belem, state of Para, have hypandria shown in figure 4. The hypandrium is relatively shorter and broader than those in the representatives of the species from southern Brazil; the lobes are angular, like those in the São Paulo flies. A strain from Soledad, Cuba, obtained through the courtesy of Mr. J. I. Townsend, has the hypandrium shown in figure 5. In the Cuban flies, the shapes of the hypandria and of the lobes are like those in the flies from São Paulo. However, the base of the bristle on the lobe is distinctly below the margin, while in the other strains examined the bristle is inserted on the margin itself (figs. 1–4). It should be noted that according to Burla et al. (1949) the insertion of the bristle found in the Cuban strain of *D. paulistorum* is characteristic of *D. willistoni.*

The flies from the Rio Grande do Sul strains were crossed to those from the São Paulo strains. The F_1 males had hypandria like that shown in figure 2. The shapes of the lobes are intermediate between those found in



FIGURES 1–5, Hypandria of different populations of *Drosophila paulistorum.* Figure 1, Osorio, Rio Grande do Sul; figure 3, Mogi das Cruzes, São Paulo; figure 2, the F_1 hybrid between the Rio Grande do Sul and the São Paulo populations; figure 4, Belem, Para; figure 5, Soledad, Cuba. The scale in figure 4, representing 100 micra, is common to all the figures.

the parental strains. Despite some variability, noticeable particularly in the hybrid flies, it is possible to distinguish without doubt the hypandria of the Rio Grande do Sul and of the São Paulo populations from those of the F_1 hybrids. An F_2 generation was obtained from these hybrids. Three clear-cut types of the lobes of the hypandria appeared among the F_2 males. Among 135 male flies investigated, the following types of hypandria were found:

	Rio Grande do Sul type	Intermediate F_1 type	São Paulo type	Total
Observed	33	71	31	135
Expected	33.75	67.50	33.75	

The observed frequencies agree with those expected if the shape of the lobes of the hypandrium is determined by a single pair of alleles without dominance (chi-square 0.406). Crossing the Rio Grande do Sul strain with that from Para results in the males having hypandria which, with respect to the length/width ratio, resemble those of the Para strain. The shape of the hypandrial lobes is intermediate between the parental strains, just as it is in the São Paulo × Rio Grande do Sul hybrids. Finally, the hybrids between the São Paulo and Para strains show a dominance of the São Paulo shape of the hypandrium; the lobes in the two strains and in the hybrids are, as expected, alike. An interesting difference between the Rio Grande do Sul and the São Paulo strains on one hand, and the Para and Cuba ones on the other, is the position of "teeth" located between the hypandrial lobes (shown in black in figs. 1–5). In the southern strains the bases of these teeth are closer together than they are in the northern strains. The hybrids between the Para and the southern strains show a dominance of the southern trait. A small F_2 generation examined suggests that this trait is also determined by a single pair of alleles.

It appears, then, that geographic strains of *Drosophila paulistorum* differ in the shape of certain parts of the genitalic armature. The genetic basis of at least some of these differences is simple. A study of the incidence of the genes which determine these differences in various populations would require collection of new material in different parts of the species' distribution area.

The author wishes to acknowledge his obligation to Professor Th. Dobzhansky for his help in the preparation of this manuscript.

SUMMARY

Strains of *Drosophila paulistorum* from three different localities in Brazil (in the states of Rio Grande do Sul, São Paulo, and Para) and from one locality in Cuba proved distinguishable by the shapes of their hypandria (figures 1–5). The Rio Grande do Sul strain differs from the others by the rounded, instead of angular, processes flanking the forceps. This difference proved to be determined by a single pair of alleles without dominance.

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This book is the best vertebrate zoology to appear in recent years. It excels in versatility of the treatment, emphasis in the selection of material, and the clear and interesting style in which the author writes. It is a bit large to be considered a textbook and unfortunately (or fortunately) it does not follow the treatment usually given the vertebrates in elementary zoology and comparative anatomy courses in American colleges. In contrast to the dull and almost meaningless detail that is so often dealt with in comparative anatomies, it is refreshing to find a book that conceives the vertebrates to be organisms that maintain and procreate themselves as individuals and as populations. In this regard a consideration of comparative embryology and of mammalian structure and function is conspicuously absent; but Professor Young promises to deal with these subjects in a separate volume. The factual material in the existing volume is up to date; for example, Westoll's interpretation of the skull bones of fishes is followed, the work of Lack on speciation in birds and of Gray's school on vertebrate locomotion is dealt with, etc. Some errors inevitably exist. Their minor nature can be indicated by noting that they involve such things as a failure to distinguish between enterocoels and schizocoels in the formation of the mesoderm in *Amphioxus*, and the crediting of a drawing of spawning *Lampertra wilderi* Gage to Gage instead of to Dean and Sumner. Many of the illustrations are original and most are of excellent quality. Professor Young is to be congratulated in having produced a book that will be constantly useful in all laboratories where vertebrates are used as material for research and for teaching.

F. J. R.